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STURMIA INCONSPICUA MEIGEN, A TACHINID PARASITE OF THE GIPSY MOTH[1]

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INTRODUCTION

Sturmia inconspicua Meigen is recognized as one of the most important parasites of the introduced pine sawfly, *Diprion simile* (Hartig) (*Lophyrus similis* Htg.). As a parasite of the gipsy moth (*Porthetria dispar* L.) it is not so well known, although its attack upon that species was recorded many years ago. As a parasite of *P. dispar* in Europe it compares favorably with other tachinids except *Phorocera agilis* Robineau-Desvoidy and *Sturmia scutellata* Robineau-Desvoidy. In certain localities it has even outranked the latter parasite. Illustrative of the periodic abundance of this species are the rearings made at Rembertow, Poland, in 1927. Approximately 200,000 *P. dispar* larvae were reared for their parasites, and from them were obtained more than 20,000 puparia of *S. inconspicua*.

This increased knowledge of the insect's importance as a gipsy-moth parasite and the establishment in the northeastern part of the United States of one of its favorite hosts, *Diprion simile*, have renewed interest in this species.

SYNONYMY

European authorities do not wholly agree upon the taxonomic status of *Sturmia inconspicua*. According to Bezzi and Stein,[3] *inconspicua* Meig. (1830), *flavoscutellata* Zett. (1844), and *cursitans* Rond. (1861) are synonyms of *bimaculata* Htg. (1838). It is not clear why these authors used *bimaculata* when *inconspicua* has priority. Probably it is an instance of common usage. *S. gilva* Htg. (1838) is regarded by them as distinct. In a later work Stein[4] still holds to his opinion as regards the distinctiveness of the two forms *bimaculata* and *gilva*, but here he recognizes the priority of *inconspicua* over *bimaculata*. Baer,[5] like Bezzi and Stein,[3] uses *bimaculata* in preference to *inconspicua*, but he considers *gilva* a synonym, or at most a variety, of *bimaculata*. In 1926 J. Villeneuve discussed the synonymy of these forms with the writer. According to him, they are all synonymous with *S. inconspicua*, *gilva* being at most a poorly marked variety.

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[3] BEZZI, M., and STEIN, P. KATALOG DER PALÄARKTISCHEN DIPTEREN. Bd. 3, p. 227. Budapest. 1907.

[4] STEIN, P. DIE VERBREITETSTEN TACHINIDEN MITTELEUROPAS NACH IHREN GATTUNGEN UND ARTEN. Arch. Naturgesch. (A) 90 (6): 57. 1924.

[5] BAER, W. DIE TACHINEN ALS SCHMAROTZER DER SCHÄDLICHEN INSEKTEN. Ztschr. Angerw. Ent. 6: 242. 1920.

GEOGRAPHICAL DISTRIBUTION

Sturmia inconspicua is common to central Europe and northern Africa. According to Fisk,[6] who refers to it under the name *Zygobothria gilva*, it is more abundant in the Mediterranean countries than in the north. In recent years, however, practically all the material collected has come from northern Europe, especially the area where pine is grown. It is probable that its regional abundance has some connection with the presence of its alternate hosts.

HISTORY OF IMPORTATION AND COLONIZATION

In 1906 the first attempt was made by the State of Massachusetts and the Federal Government to introduce the species into the United States. The colony was small and there is some doubt concerning the point of liberation. Presumably it was in the vicinity of North Saugus, Mass. During 1907–08 but few puparia of this species were received from abroad and no colonies were liberated. In 1909 more than 7,000 puparia were received from France and colonized at Wellesley, Wenham, Stoneham, Byfield, and Melrose Highlands, Mass. In addition 385 puparia were obtained from importations of *Porthetria dispar* from Belgium, Austria, and Germany, and flies were liberated in the same localities. During the following years all attempts to recover the species failed, and it is safe to conclude that the establishment of *inconspicua* from this introduction was unsuccessful.

In recent years another effort has been made to establish the species. During 1923–24 only a small quantity of material was received and no colonization attempted. In 1925, 1,886 puparia were imported from Poland, Hungary, Portugal, and Czechoslovakia. Of this material two colonies, aggregating 1,200 adults, were liberated under unsatisfactory conditions. Less than 60 puparia were received in 1926 and none of the flies was liberated. In 1927 importations totaling 15,607 puparia were received from Poland, Hungary, and Yugoslavia, and approximately 4,798 adults were liberated in Massachusetts, New Hampshire, and Rhode Island. In 1928 nearly 55,000 puparia were received from the same localities, and four colonies, aggregating 28,000 adults, were placed in Massachusetts, New Hampshire, and Rhode Island.

The first recovery of *Sturmia inconspicua* was made during July, 1929, when seven puparia were obtained from *Porthetria dispar* larvae collected at the colony site in Saugus, Mass. This colony was liberated in July, 1927.

DESCRIPTION OF STAGES

The description that follows has been drawn from a large number of specimens of both sexes reared from *Neodiprion dyari* Roh. and *Porthetria dispar* L. at the gipsy moth laboratory. The parent stock was bred from *Lophyrus pini* L. collected at Wloclawek, Poland.

[6] FISKE, W. F. PARASITES OF THE GIPSY AND BROWN-TAIL MOTHS INTRODUCED INTO MASSACHUSETTS. WHERE THEY COME FROM. WHAT THEY ARE DOING. A GENERAL SURVEY OF THE WORK. p. 39, illus. Boston. 1910.

THE ADULT

Head (fig. 1): Horizontal axis, measured at base of antennae, but little wider than at vibrissae. Front variable; average specimen at widest part measuring, in the male, a little less than and, in the female, a little more than one-third the head width. Eyes bare, bucca from one-eighth to one-fifth the eye height. Front and parafacials usually gray pollinose, the former often with a decided golden tinge that sometimes spreads over the entire face (more pronounced in the females) and bearing, in the male, a row of 10 to 12 and, in the female, from 7 to 9 bristles, the lowest and uppermost ones of which are reclinate, and those of the intermediate region decussate. Outside of and parallel with frontal row, several bristles which in some individuals give the appearance of a double row. These bristles are variable and are sometimes entirely absent in the female. In the male the inner vertical bristle large, the outer vestigial; in the female both the vertical bristles well developed, the outer one less strong. Facial ridges bristly on the lowest fourth; palpi yellow. Antennae usually black, reaching lowest fourth of face, third joint in both sexes from two to two and one-half times length of second. Base of third joint and apex of second sometimes red or yellowish, particularly in the female. Arista tapering from base to apex.

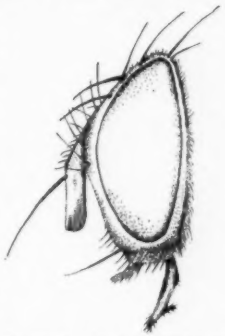

FIGURE 1.—Head of adult female of *Sturmia inconspicua*, lateral view

Thorax black, moderately to thickly gray pollinose and bearing before the suture 3 acrostichal and 3 dorsocentral bristles; behind the suture either 3 or 4 acrostichal (usually 3) and 4 dorsocentral bristles. Scutellum black at base, the apical half brownish and bearing 3 pairs of strong marginal bristles besides the decussate apical pair. Disk thickly beset with fine depressed hairs, from which arises 1 strong pair of widely separated bristles. Sternopleura usually with 4 bristles, sometimes 3 and a weaker one. Pteropleural bristle small.

Abdomen black, at least the basal half of segments II, III, IV, and a good part of the pleural and ventral regions moderately gray pollinose; segment I and the apices of the following segments shining black. Segment I with no macrochaetae; segment II with a marginal pair; segment III with a marginal row; and segment IV wholly covered except at extreme base. Sides of the second segment sometimes reddish. In the male, underneath segment III there is a small dense patch of depressed hairs similar to that found in *Zenillia eudryae* Town. and in several species belonging to the genus Sturmia. This character is absent in the female. Mid tibiae with 1 bristle on the outer front side near the middle, 1 on the forward side which often appears as an inner bristle, and 2 hind bristles. Hind tibiae thickly ciliate, with 1 longer bristle.

FIGURE 2.—Male hypopygium of *Sturmia inconspicua*: A, Rear view; B, side view

Wings hyaline, third vein usually with one strong bristle at base, sometimes a weaker one present. Apical cell open, ending far from the extreme wing tip.

Genitalia (fig. 2) small, retracted. Inner forceps shining black and tapering to slender points. When viewed from the side they are nearly straight, the basal half densely clothed with fine black hairs which are directed upward; when viewed from behind they are distinctly separated from base to apex, parallel for one-half of their length and then outwardly bowed. Outer forceps light brown, shining, wide at base, and tapering to blunt, rounded points; outer surface sparsely clothed with light-brown hairs. Anterior claspers elongate, slender, brownish, terminating in fine points; posterior claspers short, knoblike, scarcely three times as long as their apical width.

Length, moderate size flies, usually about 7 mm but sometimes as long as 10 mm.

THE EGG

Length from 0.55 to 0.60 mm, width 0.18 to 0.20 mm, depth about equal to width; transparent; moderately elongate-oval in profile, posteriorly rounded and tapering anteriorly; lower side concave. Viewed from above, surface of egg finely punctate, with distinct reticulation.

THE LARVA

Stage 1 (fig. 3, A).—Size variable. A larva 2 days old measured 1.0 mm in length and 0.33 mm in width; another larva, 3 days old, measured but 0.80 by 0.27 mm. Moderately elongate, tapering anteriorly, posteriorly rounded; skin colorless and transparent; head without spines and bearing 2 conspicuous pairs of rodlike sensory organs. On segment I is an anterior band of spines made up of 3 to 5 irregular rows, which are weakest in the pleural region. The spines of the dorsum are less numerous but stronger than those of the venter and stronger than those of any other segment except the anal segment, where the stigmatic spines are located. There is also a much weaker dorsolateral band of 2 or 3 rows commencing near the posterior border of this segment and merging into the anterior row at the pleural region. All spines directed posteriorly. Segment II banded anteriorly with 3 to 5 irregular rows of spines, posteriorly directed, and somewhat weaker than those of the dorso-anterior border of segment I. Rows more numerous in ventral region. Segments III and IV likewise banded anteriorly with 3 to 5 irregular rows of posteriorly directed spines slightly weaker than those of the preceding segment. Posterior two-thirds of segments II, III, and IV apparently naked. Segments V and VI with anterior bands of 3 to 5 irregular rows of posteriorly directed spines which are weakest in the pleural region. Posterior margins of segments sparsely clothed with incomplete rows of minute anteriorly directed spines. Segments VII and VIII with weak anterior bands consisting of from 2 to 4 incomplete rows of posteriorly directed spines which are feeble or missing in the pleural region, also posterior bands of from 2 to 4 rows of anteriorly directed spines which are but narrowly separated from the posteriorly directed spines of the segments which adjoin them. Posterior bands weak along the dorsum. Segments IX and X with incomplete anterior bands of 3 to 5 rows of posteriorly directed spines which are interrupted at the pleural region and strongest at venter. The posterior borders of these segments have more or less complete bands of several rows each of anteriorly directed spines. On segment X the posterior band is broken at venter, but midway to the suture which separates segment IX a short, though well-defined, ventral row of anteriorly directed spines can be observed. Segment XI has no well-marked anterior band of spines, although in some specimens there appears to be a weak, sparsely beset row along the suture that separates segments X and XI. Ventrally, near the middle of segment XI, an irregular row of anteriorly directed spines of varying length, widest at venter and narrowing as they approach the pleural region, where they terminate in a small patch of 5 to 8 strong spines which are of an entirely different character from those which precede them. Region adjacent to posterior spiracles sparsely beset with weak, anteriorly directed spines, inconspicuous in most part, even under high magnification. Anterior to, but directly between, the spiracles there arise 3 to 6 strong, black, anteriorly directed stigmatic spines. Opposite them a somewhat similar posteroventral patch of strong anteriorly directed stigmatic spines. Segment XI further characterized by 2 conspicuous brown sclerotized spots each equal in size to one of the posterior spiracles and situated midway between them and the ventral patch of stigmatal spines. There are also several pairs of papillae on this segment. These papillae are the usual clavate sensorial organs and are about 5 times as long as they are broad.

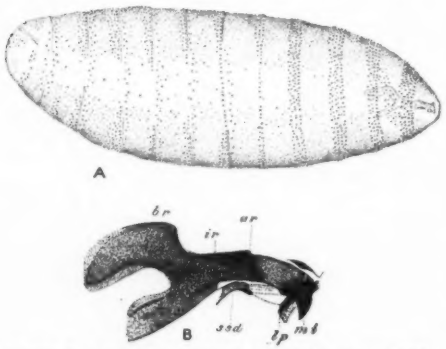

FIGURE 3.—*Sturmia inconspicua:* A, First-stage larva (× 64); B, bucco-pharyngeal armature of same (*br*, basal region; *ir*, intermediate region; *ar*, anterior region; *ssd*, sclerite of the salivary duct; *lp*, lateral plates; *mt*, median tooth)

The first-stage larva is metapneustic, the posterior spiracles opening near the apex of the last segment considerably dorsad of the longitudinal axis. Bucco-

pharyngeal armature without articulations. (Fig. 3, B.) Anterior lateral plates (*lp*) lightly pigmented and irregularly shaped; the sclerite of the salivary duct (*ssd*) large, somewhat shoehorn-shaped with a slender ventral appendage; anterior region (*ar*) with a median tooth (*mt*) directed straight downward, moderately thickened and pointed at tip, bearing on the upper half of its anterior edge a series of 4 or 5 distinct, though minute, accessory teeth. Behind the teeth and arranged in parallel formation are a number of well-defined striae. Basal half of anterior region lightly pigmented. Intermediate region (*ir*) defined on its dorsal anterior edge by a slight hump and on its ventral posterior edge by a marked indentation of the basal region. Upper wing of the basal region (*br*) abruptly curved, at its widest part nearly one and one-half times the width of the intermediate region; lower wing weakly sclerotized at apex and about one-half the size of the upper one.

Stage 2 (fig. 4, A).—Length and width variable. Average measurements 2.0 to 2.5 mm by 0.80 to 1.0 mm. Segment I with dorsal and ventral spinous areas composed, respectively, of 4 and 7 irregular rows of posteriorly directed spines. Pleural region bare. Segments II and III with complete anterior bands composed of 5 to 7 rows of posteriorly directed spines, weakest in pleural region and strongest at venter. Spines of segments I and II stronger than those of any other segments. Posterior borders of segments I and II bare. Segment III with a few weak, scattered spines in the posterior region. Segment IV with a complete anterior band composed of 5 or 6 rows of posteriorly directed spines, also an incomplete ventroposterior band of anteriorly directed spines of the same strength which end in the pleural region. Segment V with an anterior band (sometimes narrowly interrupted at dorsum) of 4 or 5 rows, which are directed posteriorly, and a posterior band of anteriorly directed spines of the same strength, which is also interrupted at dorsum. Segments VI to IX with complete bands of weak spines composed of 5 to 7 rows bordering their anterior and posterior margins. The spines of the ventral region are stronger than those of the dorsum. All spines bordering the anterior edge of segments directed posteriorly, those of posterior edge directed anteriorly. Segment X with weaker and narrower bands of 3 to 5 rows of posteriorly directed spines on its anterior margin. Spine rows of venter more numerous than those of dorsum. Posterior margin of this segment partially banded (interrupted at venter), with numerous irregular rows of anteriorly directed spines much weaker than those of the preceding segments. Segment XI with no well-defined anterior spine bands, but entire dorsum lightly beset with weak spines and the pleural and ventral regions feebly spinose. Posterior region with 5 or 6 irregular rows which vary greatly in strength and arrangement. At venter, between these rows and the suture, a conspicuous ventrolateral patch of weak, anteriorly directed spines. Extreme posterior region bare. Stigmatic spines and sclerites of anal segment, which are characteristic of the first-stage larva, not present in this stage.

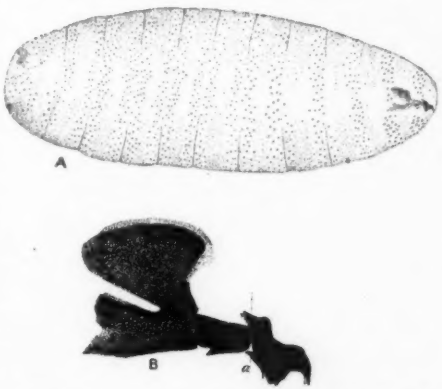

FIGURE 4.—*Sturmia inconspicua:* A, Second-stage larva (× 26); B, bucco-pharyngeal armature of same (*a*, joint separating mandibular hooks from intermediate section)

Anterior spiracles, if present, inconspicuous and difficult to locate. Posterior spiracles located, as before, on dorsal surface of anal segment and appearing more robust than those of the preceding stage.

The bucco-pharyngeal armature is shown in Figure 4, B. In this stage there is a well-marked joint (*a*), which separates the now paired mandibular hooks from the intermediate section.

Stage 3 (fig. 5, A).—Length and width variable. Average measurements 8.0 to 9.0 by 3.0 to 3.5 mm. Head bare except about the mouth, where there is a small patch of posteriorly directed spines. Two pairs of sensory organs conspicuous. Segment I, anterior edge bordered dorsally and ventrally with 6 or 7 irregular rows of short, black, posteriorly directed spines; pleural region weakly spinose (3 or 4 rows); posterior region having few weak spines, in most part outwardly directed. These spines (fig. 5, B) are nearly colorless, unusually long and hairlike, and in decided contrast to those of the anterior border. Segment II, anterior border with 7 or 8 rows of short, black, posteriorly directed spines, strongest in the dorsal and ventral regions; posterior region less thickly clothed with hairlike spines, those nearest the margin, at least, directed anteriorly. Segments III to X with complete anterior bands of 5 or 6 irregular rows of posteriorly directed spines, in most part hairlike, weakest in the pleural regions and strongest ventrally; posterior regions of these segments thickly clothed with the same type of spines, anteriorly directed; intermediate regions less densely spinose. Anal opening situated on posterior margin of segment X, on ventral side. Space around opening, which is as large as that from which one of the posterior spiracles arises, bare and entirely surrounded by black spines. Segment XI with anterior border clothed with several rows of hairlike spines, in most part posteriorly directed. Posterior extremity, ventrad of the spiracles, defined by a weak suture bordered anteriorly by a dense ventropleural band of anteriorly directed spines; immediate region either side of this band less thickly beset with weak, colorless spines. Except for the bare area adjacent to the spiracles, which is broadest ventrally, and the narrow ventral anterior border of the posterior extremity, which is nearly bare, the segment is thickly beset with anteriorly directed, short, black spines, similar to those which form the anterior bands of segments I and II. Posterior spiracles (fig. 5, C) opaque brown or black, protuberant, their stalks directed obliquely upward, about as long as broad. Each spiracle with three light brownish respiratory slits, varying somewhat in length and arrangement, located on top of well-defined ridges. In the slide mounts it is difficult, because of their irregular elevation, to obtain a good perspective of the posterior spiracles. Usually they appear as in Figure 5, C, although they are frequently distorted so that only two of the slits are visible. Anterior spiracles, if present, inconspicuous and difficult to locate.

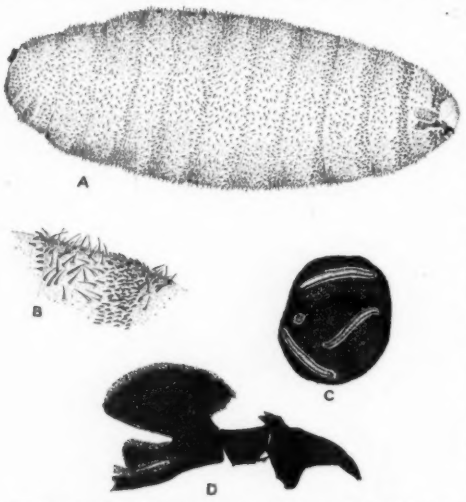

FIGURE 5.—*Sturmia inconspicua:* A, Third-stage larva (× 7.5); B, dorsal view of segment II of same, showing type and arrangement of spines; C, posterior spiracles of same; D, bucco-pharyngeal armature of same

The bucco-pharyngeal armature is represented in Figure 5, D. In this stage there are three articulations which can be readily discerned, although in some cases the segmentation between the intermediate and the anterior regions is not clearly defined. Other differences separating this stage from the preceding one are the elongation of the laterally paired mandibular hooks, the subsequent modification of the sharp angles which characterized the anterior region of the second stage, and the very distinct formation of the intermediate section.

THE PUPARIUM (fig. 6)

Length and width variable, specimens as small as 3 by 1 mm and as large as 7 by 3 mm have been noted. Usually opaque brown or black, but sometimes light

brown and semitransparent, never shining. The long hairs borne on it give a dull appearance and also favor the accumulation of dirt. Posterior spiracles (fig. 6, B and C) located just above the horizontal axis and separated at base by a distance about equal to their width, conspicuously protuberant, the stalk shining black, directed obliquely upward, about as long as broad, each spiracle and three brownish slits each located on top of a well-defined ridge. Button small, located at inner edge of spiracular plate and, in some cases, poorly defined and appearing as a minute shining black dot some distance from the edge. At the base of the tubercles which form the spiracles, below and between them, is a slight elevation with a median depression. Prothoracic spiracles inconspicuous and, if present, extremely difficult to locate on the external surface of the puparium. Anal opening large, located far below the posterior spiracles, near the posterior border of segment X.

SEASONAL HISTORY

In Europe the species passes the winter as a first-stage maggot within the cocooned larva of several species of the old genus Lophyrus (sawflies) and in the overwintering larva of *Dendrolimus pini* L. At the beginning of spring the hibernating maggot develops to maturity and, in the case of a sawfly host, escapes from the cocoon through a circular opening cut at one end. The pupal period is about two weeks long. The adult then issues, mates, and in about another two weeks attacks those hosts which are suitable for its perpetuation. The maggot of the next generation develops rapidly within the parasitized host larva, some individuals completing their larval development in six days. The adults of this generation (summer generation) appear about a month later. They behave as did their parents, mating and later ovipositing upon certain host species which are then available. As a rule it is the progeny of this generation that hibernate, although there are apparently some individuals which emerge, thereby forming a partial third generation. At the Budapest laboratory oviposition by the flies of the summer generation was obtained as late as September 12 on *Porthetria dispar* in which development had been retarded for several weeks. Their progeny did not attempt to hibernate, but completed their development, issued, and gave adults between October 20 and 27—that is, after 38 to 45 days. These adults were then mated and later successfully deposited eggs on *Plusia gamma* L. and *Noctua c-nigrum* L. (*Agrotis c-nigrum* L.).

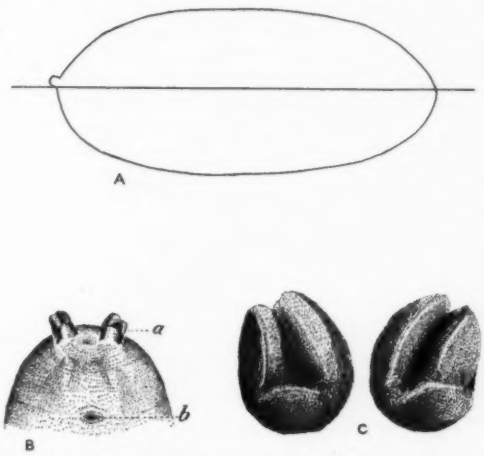

FIGURE 6.—*Sturmia inconspicua:* A, Puparium, lateral view (× 8); B, ventral view of posterior end held at an angle of 45° (*a*, stigmata; *b*, anal opening); C, posterior spiracular plates

LIFE HISTORY

MATING

As is usual among the tachinids, the males issue first. Several days elapse between the height of their emergence and that of the females. Mating of the species was readily obtained in cages measuring 14 by 16 by 36 inches. These cages were of wood frame and covered with cloth with the exception of part of the front, which was celluloid. One cage would accommodate 300 flies. In shell vials, 2 by 8 inches, and in smaller containers mating was not so easily obtained.

Usually the females are several days old before mating is effected. In other species instances have been recorded of the males mating with females whose wings were scarcely dried. Of the several thousand flies observed at this laboratory, such an occurrence has not been recorded.

According to observations made at the Budapest laboratory, the mating flies prefer the bright sunlight. It was found that when the flies were moved from the laboratory into the bright out-of-door light mating would take place at once. At the Melrose Highlands, Mass., laboratory, however, the flies apparently mated very well in the semidarkened laboratory.

The pairing of the species is usually preceded by a momentary struggle of the flies; they then settle down and remain more or less stationary for a variable length of time. Ordinarily the flies remain in coitu for several hours, and frequently those taken from the cages during the evening remain so until the following morning. In some cases, however, a pair will separate in less than an hour even though there is no obvious disturbance. Of nine pairs removed from the cages June 8, the time spent in coitu was: 1¼ hours for one pair, 4¼ hours for four pairs, and more than 5 hours for the remaining pairs.

Males that have mated once will do so a second time and the result of the second union is successful. Females seen in coitu from 15 minutes to 1 hour have been observed to separate and remate after a short time, the second mating continuing for more than 6 hours.

PREOVIPOSITION PERIOD

In 1928 adults began to issue from the first installment of imported material upon its arrival at the laboratory on May 18, and continued issuing until the middle of June.

On May 31 and June 1, 20 pairs of flies (in coitu) were taken from the cages, placed in separate containers, and their behavior noted. Dissection of some females five days after fertilization showed the uterus swollen to many times its original size and packed with minute, elongated maggots each inclosed within a thin membranous eggshell. Occasionally a marked difference in the development of the eggs within the uterus was noted, those at the anterior end being semitransparent and motionless while those at the posterior end contained fully developed maggots, some of which were active. By actual count there were 97 maggots in the uterus of one female and 102 maggots in that of another.

After the third day of mating, various host larvae were presented to the flies in an attempt to obtain oviposition. This procedure was

continued daily up to the time of oviposition, which usually did not occur until 12 to 14 days after mating. The minimum period for preoviposition was obtained from two females taken in coitu June 8 and separating after two hours. Seven days later these two flies oviposited on the penultimate-stage larvae of the sawfly *Neodiprion dyari*. In every other instance where flies of this age were used the results were negative. They would either appear frightened or show no interest whatever in the caterpillars before them.

OVIPOSITION

Oviposition has been repeatedly observed. The fly faces the host, and, with its ovipositor extended between its legs, calmly proceeds to attach an egg to the skin of the host larva. Often the fly will stand at almost the maximum distance away from the host larva, so that an active larva could easily crawl out of range. This behavior was apparent when the species was confined with *Hyphantria cunea* Drury or *Diacrisia virginica* Fab. Oviposition was frequently attempted on the rapidly moving larvae, but rarely were the flies able to deposit their eggs. As a rule, once a larva escaped, the parasite would not only fail to follow it up, but apparently lost all interest in it. With a less active species of host, especially the tenthredinids, the flies were more persistent and deposited many eggs. In certain species of this family a larva when disturbed raises the anterior portion of its body and jerks its head backwards and sideways until relieved from alarm. This behavior on the part of the species observed was futile, however, so far as *Sturmia inconspicua* was concerned. Such action may have delayed oviposition, but eventually the flies succeeded in their attack and deposited many eggs.

The fertilized female, when given preferred host material, did not exhibit the degree of excitement noted among some other tachinids. This was also true of the females that had been held back after fertilization for experimental purposes. Only rarely did any of the flies exhibit excessive eagerness to oviposit, even under what were considered to be ideal conditions.

Sensing of the host by tapping it with either the front feet or the antennal organs was not a prerequisite to attack, although the flies would often go through with these preliminaries. Motion, even of inanimate material, seemed to supply the necessary stimulus, and occasionally they would attempt oviposition on the forceps or brush as it was being withdrawn from the vial which contained them.

Oviposition was obtained at all hours of the day. Night conditions were simulated as nearly as possible, but the flies that would oviposit by day would not do so in darkness.

Apparently the flies will deposit their eggs on any part of the host. Perhaps the pleural region received the most eggs. If so, it was simply because this region was most accessible. As a rule, the eggs were laid perpendicular to the longitudinal axis of the host larva, although they were frequently found otherwise. Eggs deposited on the head capsule and on the dorsum where the integument is thickest have been noted. When they are laid on the head it is not known to a certainty that the maggots succeed in penetrating. If not, they probably die, for in no instance has any migration of the maggot been observed. A maggot hatching from an egg laid on the dorsum of *Neodiprion dyari*

or *Porthetria dispar* has no difficulty in penetrating. In other species the maggot might experience some difficulty, but no information concerning this point is available. At the Budapest laboratory, when eggs were laid on the proleg of *P. dispar*, the maggots were successful in reaching the body cavity. Eggs deposited on the sides of the thoracic segments have the best chance of survival, as the host larva is then unable to crush them. No data are available, however, which could possibly be interpreted to indicate that the fly was aware of this fact.

In confinement the number of eggs deposited on a single host is governed largely by the fly's eagerness to deposit or by the length of time that the host is subject to attack.

Sturmia inconspicua shows a preference as to its hosts. This is illustrated by the following experiment: Larvae of various stages of *Porthetria dispar*, *Malacosoma americana* Fab., *M. disstria* Hbn., *Hemerocampa leucostigma* A. and S., and *Neodiprion dyari* were placed in a cage with a number of *S. inconspicua* and allowed to remain until the next day. Subsequent examination of this material showed all the larvae free of eggs except the *N. dyari*, which were heavily infested. Other experiments were of a confirmatory nature. For instance, if *inconspicua* were placed in the same cage with *Diprion simile* and *N. dyari*, their interest would eventually center upon the latter. Furthermore, flies actively engaged in attacking *N. dyari* when transferred to *P. dispar* or *Malacosoma* spp. failed to exhibit the same eagerness to oviposit. When these hosts were replaced by *N. dyari*, the flies resumed their former interest.

LONGEVITY

Under laboratory conditions *Sturmia inconspicua* is a hardy species, there being little difficulty in keeping the flies in good condition. Individuals well cared for by daily feeding and cleansing of containers lived for 4 or 5 weeks. An instance was observed in which two females, removed from the mating cages, in coitu with males, on May 31 and used daily in reproduction experiments, lived for 42 days. A case of one female fly living for 112 days has been recorded at the Budapest laboratory.

PARTHENOGENESIS

Virgin females showed little interest in the usual hosts and, although they occasionally attempted oviposition, they were never successful. Furthermore, dissection of the flies revealed no egg development whatever. Parthenogenesis is therefore unlikely. The fact that no infertile eggs were deposited by any of the flies is interesting. In *Carcelia laxifrons* Vill., *Phorocera agilis*, and other tachinids, even though the female is unfertilized, the eggs will descend into the uterus and eventually be deposited.

DEVELOPMENT OF THE LARVA

From egg deposition to the complete disappearance of the maggot within the host is a matter of 5 to 10 minutes only. The maggot hatches immediately from one end of the egg and bores its way into the caterpillar, the chorion of the egg collapsing simultaneously with the exit of the maggot. Its progress is then arrested and it soon

becomes enveloped within an integumental funnel caused by an ingrowth of the body wall of the host. Apparently there is no attachment to any of the tracheae, but the maggot obtains its supply of air directly from the exterior by way of the funnel, in which its posterior stigmata are tightly fitted. This funnel is cone-shaped, the basal portion being highly sclerotized, dark brown to black, and gradually fading out into a lighter shade anteriorly until it becomes hyaline and difficult to see. The entire first and second stages and a part of the third stage are spent in this fixed position. The maggot then leaves its funnel and wanders freely in the body of the caterpillar. It is thought to remain thus for at least a day. This belief is strengthened by the fact that, when nearly mature third-stage maggots are dissected out alive and are allowed to remain on a watch glass with the dead host material, they will feed and complete their development and produce perfect adults. Under such conditions several maggots have lived for more than a day before transforming to pupae.

Sometimes the attack of the parasite does not bring about any definite reaction from the host larva other than a slight contraction of the segments at the moment of oviposition. Certain individuals seem to be totally unaware of the entrance of the maggot and display no apparent discomfort. Others move constantly about, throwing back the head and trying to dislodge the burrowing maggot. A parasitized larva, when exposed to a second attack one to seven days later, behaves precisely as does one suffering its initial attack. Apparently there is no recognition of its enemy.

The time required for the growth of the different larval stages is variable. Over 100 penultimate-stage larvae of *Neodiprion dyari* were parasitized in one day. Dissections of this material, together with that obtained from other host species (*Malacosoma disstria*, *M. americana*, and *Porihetria dispar*), showed a great disparity in larval development. First-stage maggots were found, either alone or in company with more fully developed ones, in larvae that had been parasitized up to 12 days; second-stage maggots were found in material parasitized from 6 to 12 days; and third-stage maggots in material parasitized from 6 to 27 days. The average duration of the larval stages of 104 individuals was 13.9 days. Usually it was not until the sixth day that any stage other than the first was found, although a few individuals reached maturity in this time. There seems to be no extra development of the maggot in the uterus of females that have withheld their eggs for an extended period. Some of the flies were held in reserve and not allowed to oviposit until 27 days after fertilization, but their progeny did not develop any more rapidly than those that were treated normally. From all the available data a mean of 3 to 6 days has been calculated as best suited to the larval development in each stage. These figures will account for at least 90 per cent of the larvae observed. It is not believed that an equal time is spent in each stage; in fact, there is some evidence that the second stage is shorter than either of the other two.

THE PUPAL PERIOD

Rarely does the maggot form its puparium within the host. Usually it cuts its way through the end of the cocoon or, in the case of a lepidopteran, tears its way through the skin of the host larva, crawls

a short distance away, and pupates. In the rearing trays the parasites often formed their puparia in the excrement and other débris. Owing to this habit and also because of the spiny nature of their skin, to which all small particles will adhere, the puparia are often mistaken for other matter and thus are easily overlooked.

The duration of the pupal stage of the summer generation was found to average about 10.5 days. A rare exception was a minimum of 7 days. The maximum time recorded was 18 days, but no doubt this could be extended almost indefinitely by lowering the temperature. The time spent in the puparium seems to depend somewhat on the sex of the individual. Of the 68 flies studied, 32 males averaged 10.9 days and 36 females averaged 11.3 days.

HIBERNATION

Sturmia inconspicua hibernates as a first-stage maggot in the cocooned larva of *Lophyrus pini* and in the overwintering larva of *Dendrolimus pini*. At the Budapest laboratory it has been observed that when the cocoons of *pini* are opened, and the overwintering larvae removed, those which are parasitized may be readily detected by the protuberance caused by the heavily sclerotized breathing funnel of the parasite. Often this structure is surrounded by a dark brown scab, and close examination reveals a minute hole through which the posterior spiracles of the parasite maggot are discernible. Communicating with the outside in this way, the Sturmia larva passes the winter in a well-defined sac which is attached to the sides of the funnel.

At the Melrose Highlands laboratory Sturmia-infested overwintering hosts (*Diprion simile* and *Neodiprion lecontei* Fitch) were dissected in October and in February of the next year, and without exception first-stage *inconspicua* maggots were found encysted beneath the skin of the host larva at their point of entrance, in a manner as described above.

SUPERPARASITISM

Superparasitism is often encountered in dissection work. Several examples which may be of interest are as follows: One *Porthetria dispar* larva parasitized 6 days contained one second-stage and one third-stage maggot; one larva of *Neodiprion dyari* parasitized 6 days contained one first-stage and four second-stage maggots in various stages of development; one *P. dispar* larva parasitized 10 days contained one second-stage (newly molted) and three third-stage maggots; one *N. dyari* parasitized 12 days contained one first-stage and one third-stage maggot.

This excessive parasitism is not always advantageous to the parasite. Many of the *Neodiprion dyari* larvae were infested with more than one parasite, yet the actual rearing of two parasites from one individual was not recorded. Evidently, in this host at least, it is the one parasite best fitted that survives. From *Porthetria dispar*, *Malacosoma americana*, and *M. disstria* several parasites have frequently been obtained from a single individual. From the Budapest laboratory Muesebeck reports three to five parasites from a single *P. dispar* larva as common, and as many as seven have been obtained. With these species it is probable that the number of *inconspicua* in each caterpillar is limited only by the size of the host.

HYPERPARASITES

According to Sitowski,[7] *Sturmia inconspicua* suffers from two hyperparasites in Poland, (*Hemipenthes*) *Anthrax morio* L. and *Argyramoeba varia* Fab. The parasitism by the former is sometimes severe. At the gipsy moth laboratory there is no record of any secondary parasite having been reared from material imported from Europe.

COMPETITION WITH OTHER PARASITES

This important phase of parasitism has not been studied in this country with sufficient throughness to warrant anything more than a general statement. In Europe, at the Budapest laboratory, some of the most interesting observations on the competition occurring between *Sturmia inconspicua* and other parasites have been made by C. F. W. Muesebeck and his assistants. These observations[8] show that—

* * * *Sturmia inconspicua* is able to complete development more quickly and thus win out in competition with two of the most common of our central European parasites of *Porthetria dispar*. When eggs of Sturmia are laid on *dispar* larvae that were parasitized as many as 8 days previously by *Phorocera agilis* or 5 days previously by *Tachina larvarum* L. it is usually the *inconspicua* larvae which first complete development and form puparia. *S. inconspicua*, when developing in otherwise parasite-free stock, requires from 6 to 20 days to form puparia, whether or not more than one *inconspicua* larva is present. *P. agilis* requires from 17 to 26 days and *T. larvarum* from 9 to 20 days under the same conditions. Since the average *P. agilis* larva requires more time than the average Sturmia, it is easily understood why *inconspicua* larvae issue first, but the case of *T. larvarum* is different. *T. larvarum*, on an average, requires no more time than *S. inconspicua*, most of its larvae forming puparia on the eleventh day in the laboratory. Nevertheless, Sturmia regularly wins over this species in competition, and it seems as if the presence of *inconspicua* larvae must have some retarding influence on those of *T. larvarum*. Later in the season *inconspicua* completes development in the larval stage very much more rapdily than *T. larvarum*. Parasite-free *dispar* larvae attacked by *T. larvarum* on August 30 produced no puparia until October 2, 33 days later, whereas *S. inconspicua* needed but from 9 to 20 days in larvae attacked September 21. In the summer, though, at the regular *dispar* season, the two species develop separately in about the same time. When, on the other hand, 30 *dispar* were attacked first by *S. inconspicua* and later by *P. agilis*, probably only the larvae which would never have produced *inconspicua* puparia or which supported both species produced those of *agilis*. Thirty *inconspicua* puparia were reared from this lot, all but one of which issued before a single one of the *P. agilis* puparia which were obtained.

From the foregoing it is evident that, because of its rapid development, *Sturmia inconspicua* is well able to hold its own even when competing against two of the most important parasites of *Porthetria dispar*.

In the United States *Porthetria dispar* has no native parasites worth mentioning. Of the introduced parasites that have been successfully established, only three, which attack the larger caterpillars, will be of interest here. These are *Compsilura concinnata* Meig., *Sturmia scutellata*, and *Phorocera agilis*, tachinid flies from Europe. The effect on these species of the establishment of *S. inconspicua* is problematical. So far as Compsilura is concerned, it is probable that an intensive competition would result in which Compsilura would be the loser. Should this prove to be the case, the status of

[7] SITOWSKI, L. DO BIOLOGJI PASORZYTOW BORECZNIKA (LOPHYRUS LATR.). (SUR LA BIOLOGIE DES PARASITES DE LOPHYRUS LATR.) Rocz. Nauk Rolnicz. 14: 1–25, illus. 1925. [Original in Polish; title in both Polish and French. Summary in German, Zur Biologie der Lophyrusparasiten, p. 22–25.]

[8] Unpublished notes at the Budapest laboratory.

Compsilura would not be materially affected because of its extraordinary ability to adapt itself to other hosts. On the other hand, *S. scutellata* and *P. agilis* are peculiar to *P. dispar;* moreover, their development is comparatively slow. For these reasons it is probable that on occasions of periodic abundance of *inconspicua* they would indeed suffer. It has often been stated that the natural control of *P. dispar* in the European forests is due, in part, to the large number of species that are parasitic upon it. Here the struggle for supremacy, or perhaps for the perpetuation, of the parasitic species themselves, is as acute as in the United States, if not more so, and yet these species persist. It is safe to predict that the establishment of *inconspicua* in this country would in no way interfere with the continuance of the other species.

The parasites of *Diprion simile* have been recorded by Middleton.[9] Mention is made of seven hymenopterous parasites and one tachinid, *Phorocera claripennis* Macq. (*Exorista petiolata* Coq.). Two species of Hymenoptera, *Monodontomerus dentipes* Boheman and *Ephialtes* (*Itoplectis*) *conquisitor* Say, and two tachinids, *Phorocera claripennis* Macq. and *Winthemia quadripustulata* Fab., have been reared at the gipsy moth laboratory. In no case were these parasites abundant. From collections of approximately 1,000 larvae only five individuals were parasitized, and it seems unlikely that *inconspicua* would find much competition in the United States as regards this host.

It is difficult to conceive of any instance in which the native parasites would suffer because of *Sturmia inconspicua.* Certainly other species besides *Porthetria dispar* and *Diprion simile* will be attacked, and the parasites peculiar to them must suffer the competition of *inconspicua.* It is by no means certain, however, that such competition would jeopardize the species so much as do numerous other factors which exert their influence entirely independent of host or parasite. The author knows of no instance in which a foreign tachinid has usurped the position of a native one. *Compsilura concinnata* is a far more adaptable species than *S. inconspicua;* yet, with all its reputed aggressiveness, there is little indication that its presence has in any way proved detrimental to its native competitors.

HOSTS

A list of the European hosts of *Sturmia bimaculata* Htg. (=*S. inconspicua* Meig.) prepared by Baer [10] includes the following species: *Dendrolimus pini* L., *Lymantria* (=*Porthetria*) *dispar* L., *L. monacha* L., *Panolis griseovariegata* Goeze, *Stauropus fagi* L., *Lophyrus pini* L., *L. similis* Htg., *L. pallidus* Klug, *L. virens* Klug, *L. frutetorum* Fab., *L. socius* Klug, *L. rufus* Klug, *L. polytomus* Htg., and *L. laricis* Jurine.

At the Budapest laboratory *Sturmia inconspicua* has been reared from several additional hosts, which are as follows: *Trichiocampus viminalis* Fall., *Pygaera pigra* Hufn., *Plusia gamma* L., *Agrotis c-nigrum* L., and from two unidentified tenthredinids.

Other host species, exclusive of *Porthetria dispar* and *Diprion simile* Htg., which have been reared at the Melrose Highlands laboratory

[9] MIDDLETON, W. THE IMPORTED PINE SAWFLY. U. S. Dept. Agr. Bul. 1182, p. 16, illus. 1923.
[10] BAER, W. Op. cit.

are *Malacosoma americana* Fab., *M. disstria* Hbn., *Hemerocampa leucostigma* A. and S., *Hydria undulata* L., *Alypia octomaculata* Fab., *Neodiprion dyari* Roh., and *Neodiprion lecontei* Fitch.

SUMMARY AND CONCLUSIONS

Sturmia inconspicua Meigen is an effective European parasite of the gipsy moth, *Porthetria dispar* L. It is also the most important European tachinid parasite of the introduced pine sawfly, *Diprion simile* Htg., and because of the recent introduction of this species in the Northeastern States the establishment of S. *inconspicua* should prove of decided benefit.

European authorities do not wholly agree upon the taxonomic status of *Sturmia inconspicua*, but according to J. Villeneuve the form treated in this paper is that species.

Sturmia inconspicua is indigenous to central Europe and is commonly found in northern Africa. The first attempt to colonize the species in the northeastern part of the United States was in 1906. Between 1907 and 1910 other introductions were made, but during the following years all attempts to recover the species failed. In more recent years (1923 to 1925, inclusive) large colonies were liberated and in 1929 the first recovery was made.

Superficially, the adult bears a general resemblance to the common house fly, but on critical examination it will be found to possess certain characters that are typical of the genus Sturmia, family Tachinidae. The eggs are grayish and about 0.5 mm in length. The first-stage larva is white, more or less spinose, and characterized chiefly by the presence of stigmatic spines and sclerotized areas on the posterior segment. The bucco-pharyngeal armature is without articulation. The second-stage larva appears less spinose and lacks the two principal characteristics of the preceding stage. The bucco-pharyngeal armature has one articulation. The third-stage larva is characterized by a clothing of spines and long hairs and protuberant posterior spiracles. The bucco-pharyngeal armature has two articulations. The puparium is brownish black, averaging 4 or 5 mm in length, and has tuberculate spiracles each furnished with three spiracular slits.

The species passes the winter as a first-stage maggot within the cocooned larvae of several species of sawflies of the old genus Lophyrus and possibly in some lepidopterous pupae. Completing its development in the spring of the year, the maggot issues, forms its puparium, and in about two weeks the adult emerges. There is a second generation, the species being able to develop on a number of hosts. It is the progeny of this generation which hibernate.

The species will readily mate in confinement. Ordinarily the flies remain in coitu several hours. Males frequently remate and the result of the second union is successful.

The preoviposition period is variable, 12 to 14 days usually being required.

The fly attaches its eggs to the skin of the host larva. In confinement the number of eggs deposited on a single caterpillar is governed largely by the eagerness of the individual to oviposit or by the length of time that the host is subject to attack.

Under laboratory conditions the flies will live for 4 or 5 weeks. An instance of one female living for 112 days has been recorded.

The species is believed not to be parthenogenetic.

The average duration of the entire larval stage is about 13.9 days, although certain individuals will mature in 6 or 7 days. The duration of the pupal stage averages about 10 days.

Superparasitism is frequent, as many as seven individuals having been obtained from a single caterpillar.

In Europe two species of hyperparasites attacking *S. inconspicua* are known, but none has been recorded from the United States.

Competition with other parasites is certain to occur, but because of the rapid development of *S. inconspicua* it will probably not suffer in this respect.

In addition to *Porthetria dispar* and *Diprion simile*, *Sturmia inconspicua* has been reared from many other host species, seven of which are native to the United States.

CORRELATIONAL AND ALLIED STUDIES OF THE PROTEIN CONTENT, WATER ABSORPTION, LOAF VOLUME, AND LOAF WEIGHT OF TWO SERIES OF HARD RED SPRING WHEATS[1]

By L. R. WALDRON, *Agronomist (Plant Breeding)*, and C. E. MANGELS, *Cereal Chemist, North Dakota Agricultural Experiment Station* [2]

INTRODUCTION

This paper presents a comparison between two series of hard red spring wheats. One series consisted of wheats diverse in genetic origin but grown under uniform conditions of soil and soil treatment. The second series consisted of a single variety grown on the plots of a rotation and fertility experimental project, located on a single soil type (Fargo clay) but subjected annually to diverse previous treatments relative to crop sequence and soil fertilization. This paper is concerned with the behavior of the two series in regard to the means, coefficients of variability, correlations (both total and net), and regression equations relative to the characters of (1) protein content, (2) water absorption of flour, (3) volume of loaf, and (4) weight of loaf.

REVIEW OF PREVIOUS INVESTIGATIONS

A number of statistical studies, emphasizing correlation results in particular, have been published on wheat for milling and on flour during the past decade. The studies reported, however, have not usually compared different series of data with their appropriate statistical constants. No doubt this is largely due to the fact that wheat and flour data have generally accumulated in connection with other experimental work and have been used only secondarily for statistical studies. Studies of variation between groups of data have thus not been possible because of limitations in number and suitability. Attention will be paid to such a comparison in this paper.

The first important studies on wheat and flour were those of Zinn (*13*)[3] who used the published data from a number of experiment stations. Zinn evidently appreciated the limitations of the data with which he worked and called particular attention to the importance of their proper grouping.

Bailey and Hendel (*1*) in their studies on the relation between protein content and test weight per bushel called attention to the necessity of grouping data by crop years, and this point was emphasized also by Mangels and Sanderson (*11*) and by Mangels (*8*).

Because of the importance of protein content as a price factor, particular attention has been given to the protein-loaf volume relation. A summary of protein-loaf volume correlation coefficients obtained by different investigators for hard red spring wheats is given in Table 1.

[1] Received for publication Jan. 26, 1932; issued September, 1932.
[2] The authors acknowledge permission to use the data from the rotation and fertility plots kindly given by Dr. H. L. Walster, chairman of the Agronomy Department and in charge of the rotation and fertility project; also their indebtedness to Dr. A. E. Treloar and his coworkers for the use of material from their study prior to its publication.
[3] Reference is made by number (italic) to Literature Cited, p. 230.

TABLE 1.—*Protein-loaf volume correlation coefficients obtained by different investigators from hard red spring wheats*

Investigator	Description of wheat	Coefficient
Zinn (*13*)	North Dakota commercial varieties	[a] −0.099
	North Dakota pure lines	.302
	Minnesota commercial varieties	.239
	Minnesota pure lines	.547
	Montana commercial varieties	.345
Mangels (*8*)	North Dakota miscellaneous named varieties	[b] .334
Bailey and Sherwood (*2*)	Diverse wheats from Minnesota, North and South Dakota, and Montana.	.271
Hayes, Immer, and Bailey (*6*)	Diverse varieties and hybrid selections	[c] .280
Coleman, Dixon, and Fellows (*3*).	Varieties diverse as to locality and variety	[d] .432
Larmour (*7*)	Standard commercial varieties	[e] .280
Harris (*5*)	Marquis wheat of the 1929 Saskatchewan crop	[f] .670
Treloar et al. (*12*)	Commercial samples milled by Minnesota State testing mill	[g] .490

[a] Zinn evidently included durum wheats which accounts for the negative coefficient. Recalculation of data for the hard red spring wheats only by Mangels (*8*) gave a positive coefficient of 0.523.
[b] Weighted average of 1,807 samples for 11 crop years separately calculated. Maximum coefficient 0.547, minimum coefficient −0.064.
[c] Weighted average of coefficients for 4 crop years separately calculated. Maximum coefficient 0.50. Each milled sample consisted of a mixture of 4 wheats, of the same kind, grown at 4 Minnesota localities.
[d] Average of coefficients from crops of 1923 and 1924.
[e] Three crop years, 1926, 1927, and 1928. Bromate baking formula gave coefficient of 0.711.
[f] Bromate formula gave a coefficient of 0.857.
[g] Weighted average of 332 samples for the 6 years, 1921–1926.

The correlation coefficients between protein and loaf volume are not usually of high order. With the exception of Zinn's comparison of pure lines and commercial varieties of North Dakota and Minnesota spring wheats, no direct comparisons are found between different groups of samples. Most of the correlation coefficients reported were obtained from groups of samples diverse within the group as to variety, and often also in other respects. The highest coefficient is that reported by Harris (*5*) for a group of samples consisting only of Marquis wheat secured from the 1929 Saskatchewan crop.

With the exception of the attention given to the subject by Treloar and others (*12*), comparatively little emphasis has hitherto been placed on the regression coefficients, in studies of the relationship of loaf volume to protein content. It has been assumed that a uniform relationship exists between the degree of correlation and the volume of the loaf. The relationship of this kind is not uniform but shows decided variations. The prediction value, deducible from the relationships of protein and volume, is decidedly affected by the deviations among the variates as shown by the regression coefficients and even then it may be necessary to consider additional variables, which find expression in the partial regression coefficients. This subject will receive further attention.

METHODS

The two series of wheats previously mentioned covered four years of cropping. One series, which consisted of wheats of somewhat diverse genetic origin was grown in the wheat-breeding nursery under uniform conditions each season. This series is designated as "nursery." The second series, which consisted of a single variety of wheat (Ceres) grown each year on a number of rotation and fertility plots diverse as to crop sequence, soil preparation, and use of fertilizers, is designated as "plots."

Since both series were grown at the North Dakota Agricultural Experiment Station, not more than one-half mile apart and also on the same type of soil (Fargo clay), they were subjected to similar climatic conditions.[4]

Three of the characters already mentioned are quantitative—protein, loaf volume, and loaf weight. The fourth character, water absorption, is expressed quantitatively, but the quantity used is estimated.

The flours used in all these studies were 75 per cent patents, prepared on a small mill. The baking tests were made according to the procedure recommended by the American Association of Cereal Chemists, using a variable absorption.

STATISTICAL CONSTANTS AND COEFFICIENTS OF VARIABILITY

The means, standard deviations, and coefficients of variability for the two series of wheats for the four years are shown in Table 2.

TABLE 2.—*Statistical constants secured from two diverse series of wheats, from the nursery and from the plots, 1927–1930*

MEANS

Year	Nursery or plots	Protein of flour [a]	Water absorption of flour	Loaf weight	Loaf volume	Variates
		Per cent	*Per cent*	*Grams*	*Cc*	*Number*
1927	Nursery	13.1±0.06	63.3±0.17	136.4±0.20	415.0±2.11	76
	Plots	10.4± .06	61.1± .13	135.4± .22	373.0±1.29	51
1928	Nursery	14.8± .09	61.2± .13	131.1± .24	454.0±4.92	59
	Plots	12.2± .09	55.2± .04	129.0± .18	484.0±4.89	70
1929	Nursery	10.8± .07	57.1± .14	133.3± .18	410.0±2.95	81
	Plots	12.1± .08	60.7± .05	136.0± .15	415.0±1.35	80
1930	Nursery	13.4± .07	58.5± .10	131.8± .25	466.0±4.23	58
	Plots	13.1± .10	63.7± .05	137.4± .10	464.0±2.29	58

STANDARD DEVIATIONS

1927	Nursery	0.75±0.04	2.22±0.12	2.58±0.14	27.1±1.49	
	Plots	.66± .05	1.37± .09	2.29± .15	13.6± .91	
1928	Nursery	1.00± .06	1.52± .10	2.69± .17	55.6±3.48	
	Plots	1.09± .06	.47± .03	2.20± .13	60.2±3.46	
1929	Nursery	.93± .05	1.88± .10	2.32± .12	39.1±2.09	
	Plots	1.01± .05	.69± .04	1.91± .10	17.8± .95	
1930	Nursery	.76± .05	1.18± .07	2.81± .18	47.7±2.99	
	Plots	1.14± .07	.61± .04	1.13± .07	25.9±1.62	

COEFFICIENTS OF VARIABILITY (IN PERCENTAGE)

1927	Nursery	5.70±0.31	3.50±0.19	1.89±0.10	6.55±0.36	
	Plots	6.37± .43	2.24± .15	1.69± .11	3.64± .25	
1928	Nursery	6.74± .42	2.48± .16	2.05± .13	12.25± .87	
	Plots	8.86± .51	.85± .05	1.71± .10	12.43± .72	
1929	Nursery	8.62± .46	3.29± .18	1.74± .09	9.54± .51	
	Plots	8.38± .45	1.14± .06	1.41± .08	4.28± .23	
1930	Nursery	5.65± .36	2.01± .13	2.13± .13	10.24± .65	
	Plots	8.73± .55	.96± .06	.82± .05	5.58± .35	

[a] Protein determinations were made with the water content calculated to a uniform basis.

The nursery group of wheats varied in its constituents somewhat from year to year. During the first three years a considerable proportion of the wheats were hybrid selections of the cross Marquis × Kota, sibs of Ceres. The remaining wheats were named varieties and selections from various crosses. The Marquis × Kota hybrids showed less variability in protein content than did the other wheats,

[4] In 1928 the two lots were affected differently by rain after cutting and previous to threshing. (Table 4.)

but a decrease in the proportion of these wheats for 1930 did not result in a relative increase of variability of protein for that year. Any increase in variability that may have occurred from a diminution in number of Marquis × Kota hybrids must have been offset by decreases from other causes.

PROTEIN CONTENT OF FLOUR

Protein content may be measured with a relatively higher degree of accuracy than other characters, and on account of its importance will be discussed separately.

The average unweighted protein content for the two series for the four years was 13 per cent for the nursery and 12 per cent for the plots. This very appreciable difference may be due wholly or in part to the previous cropping of the nursery wheats. This was as follows:

Year	Previous cropping
1927,	summer-fallowed sweetclover turned under.
1928,	flax preceded by sweetclover turned under.
1929,	oats preceded by sweetclover turned under.
1930,	cucurbits preceded by wheat, flax, and summer-fallowed sweetclover turned under.

Sweetclover preceded the wheat in all cases, but immediately only in 1927. The 1928 crop of oats carried a heavy growth of straw, and unavoidably a considerable amount of stubble was plowed under. There seems to be no reasonable doubt but that the nitrogen available to the 1929 wheat crop was greatly influenced by the crop of oats. The probable effect of the preceding oat crop on the protein content of the 1929 nursery wheat is indicated by the protein present for the years other than 1929. The average percentages of protein for the years 1927, 1928, and 1930, as compared with those for 1929 are as follows:

	Nursery	Plots
Average for 1927, 1928, 1930	13.8	11.9
Average for 1929	10.8	12.1
Difference	3.0	−0.2

Data from rotation plots (Mangels (*9*)) show that a preceding crop of sweetclover on Fargo clay soil increases the protein content of the succeeding wheat crop. Further evidence of the influence of preceding crops is shown by the protein content of the Ceres samples from the nursery samples and the Ceres averages for the plots. These comparisons are given in Table 3.

TABLE 3.—*Protein content of Ceres wheat when grown in the nursery and in the plots, compared with the protein content of all nursery wheats*

Year	Protein content of all wheats grown in nursery (1)	Protein content of Ceres grown in—		Differences between—	
		Nursery (2)	Plots (3)	1 and 2	2 and 3
	Per cent	*Per cent*	*Per cent*		
1927	13.1	12.9	10.4	0.2	2.5
1928	14.8	14.9	12.2	.1	2.7
1929	10.7	10.2	12.1	.5	1.9
1930	13.4	13.1	13.1	.3	0
Average	13.0	12.8	12.0	.28	1.78

The protein of Ceres in the nursery samples is much closer to the general nursery average than to the average of the plots series consisting entirely of the Ceres variety.

The means of the characters other than protein are very close together. The unweighted means of water absorption, loaf weight, and loaf volume for the four years were 60, 133.2, and 436, respectively, for the nursery, and 60.2, 134.5, and 434 for the plot wheats.

Attention should be called to that portion of Table 2 showing the coefficients of variability. The comparative behavior of protein under modified conditions indicated previously is shown in the present instance. To give a clear expression to the relative differences of behavior of this character in comparison with water absorption, loaf weight, and loaf volume, the differences of variability calculated from Table 2 are shown in Table 4.

TABLE 4.—*Differences of the coefficients of variablity between the nursery and plots from Table 2 for the four characters indicated, 1927–1930*

[When the ratio values (*R*) are 3 or more the differences may be considered significant]

Year	Protein content [a]	Water absorption	Loaf weight	Loaf volume [a]
1927	−0.67±0.53; *R*=1	1.26±0.24; *R*= 5	0.20±0.15; *R*=1.3	2.91±0.44; *R*=7
1928	−2.12± .66; *R*=3.2	1.63± .17; *R*=10	.34± .16; *R*=2.1	[b]−.18±1.13; *R*=0.2
1929	.24± .64; *R*= .3	2.15± .19; *R*=11	.33± .12; *R*=2.8	5.26± .56; *R*=9
1930	−3.08± .66; *R*=4.7	1.05± .14; *R*= 7	1.31± .14; *R*=9.4	4.66± .74; *R*=6

[a] Minus differences indicate that the variability of the plots is more than that of the nursery samples.
[b] The slightly greater variability in loaf volume for the plots in 1928, in sharp contrast to that obtained for the other years, may easily be due to a disturbing element of that year. The wheat from the plots was subjected to heavy rains while in the shock, whereas the nursery samples were subjected to little or no extraneous moisture before threshing.

Considering the coefficients of variability for proteins, one finds that the variability of the plots is greater in three of the four years. In two of these three years (1928 and 1930) the variability of the protein content from the plots is significantly the larger.

A closer study of the data on protein content shows that the nursery samples exhibited greater variability than would be expected from a group of Ceres samples taken at random. In 1927 and 1928 more than half of the nursery samples were sibs of Ceres, resulting from the same cross, while the remainder were miscellaneous in character. The coefficients of variability for these two groups for the two years were calculated and are shown in Table 5.

TABLE 5.—*Coefficients of variability for protein content of nursery samples that were sibs of Ceres as compared with those that were miscellaneous in character, 1927 and 1928*

Kind of samples	Variability of protein content in—	
	1927	1928
Miscellaneous	8.53±0.80	7.51±0.78
Sibs of Ceres	3.41± .23	3.84± .24
Difference	5.12± .83	3.67± .82

The protein content of the miscellaneous wheats when grown under uniform conditions is seen to be decidedly more variable than the Ceres sibs similarly grown. However, the Ceres variety, grown on the rotation and fertility plots, shows more variability among the different samples than do the samples from the nursery.

Another comparison may be made by securing differences between the coefficients of variability of the protein content of the Ceres samples from the plots and of the Ceres sibs from the nursery, for 1927 and 1928. The values are shown in Table 6.

TABLE 6.—*Coefficients of variability for protein content of Ceres samples from the plots as compared with samples from nursery Ceres sibs, 1927 and 1928*

Kind of samples	Variability of protein content in—	
	1927	1928
Ceres from plots	6.37±0.43	8.86±0.51
Ceres sibs from nursery	3.41± .23	3.84± .24
Difference	2.96± .49	5.02± .56

The evidence for the greater variability of the protein content of the samples from the plots is very definite for three of the four years. For 1929, when wheat followed oats, the difference, which is negative, is not significant. In this case the nursery samples had a relatively high standard deviation associated with a comparatively low mean.

So far as the present studies show, the preparation of the soil, crop sequence, and use of fertilizers, generally, have a greater influence on the protein content of wheat than do differences in genetic origin of wheat. Differences in protein due to genetic origin, while important, evidently are not responsible for as much variation as differences in soil fertility.

LOAF VOLUME, LOAF WEIGHT, AND WATER ABSORPTION

The means of loaf volume, loaf weight, and water absorption show no uniformity as to differences in value between the nursery and the plots, but the coefficients of variability are almost uniformly larger for the nursery samples as shown in Table 4. Only one exception is found in the 12 possible comparisons. The coefficient of variability for loaf volume for the plots for 1928 is slightly the greater, but this is probably due to sprout damage in the rotation plot samples. In 8 of the 12 cases, the differences are strikingly significant.

A distinct difference in variability is found between protein and the other three characters. Protein content responds readily to soil differences, whereas water absorption, loaf weight, and loaf volume respond to varietal differences. Of the three characters, loaf weight, loaf volume, and water absorption, loaf weight responds least and water absorption most, to varietal differences.

CORRELATION DATA

Correlation coefficients were computed between the variables for the two series. In addition to the total correlation, partial correlations were also calculated. Evidently very little use has been made

of partial correlation coefficients when studying wheat and flour data. These data seem to offer an excellent field for the application of the partial correlation. Certainly the problem allows closer approach when, for example, a direct relationship can be established between protein and loaf volume without the interference of such variables as water absorption and loaf weight, which for this purpose can be made constant.

The entire series of partial correlations are presented. A number of these may possess only minor interest, but they are listed since the general problem demanded their calculation. The coefficients of the zero, first, and second order are shown in Table 7. The weighted means of the correlation coefficients, calculated by the method of Fisher (*4, p. 170*), are presented.

TABLE 7.—*Total and partial correlation coefficients and weighted averages secured from two diverse series of wheats, from the nursery and the plots, calculated from the four characters shown, 1927–1930*

[Correlations given in columns 4, 5, and 6, represent values when element, or elements, at head of the columns was held constant]

PROTEIN-VOLUME

Year	Nursery or plot	Zero order	Loaf weight	Water absorption	Weight and absorption	Variates
1927	Nursery	0.506	0.496	0.508	0.480	76
	Plots	.683	.716	.683	.745	51
1928	Nursery	[a].351	.350	[a].262	[a].155	59
	Plots	.712	.616	.711	.616	70
1929	Nursery	[a].322	[a].307	[a].337	[a].298	81
	Plots	.674	.684	.666	.676	80
1930	Nursery	[a].583	[a].430	[a].578	[a].367	58
	Plots	.850	.853	.848	.852	58
Average, nursery		.441	.398	.427	.337	[b]274
Average, plots		.735	.722	.732	.726	[b]259

PROTEIN-WEIGHT

Year	Nursery or plot	Zero order	Loaf volume	Water absorption	Volume and absorption
1927	Nursery	−0.124	−0.044	−0.239	−0.147
	Plots	−.125	−.316	−.221	−.456
1928	Nursery	[a]−.056	.047	−.278	−.182
	Plots	−.488	−.200	−.486	−.199
1929	Nursery	[a]−.101	[a]−.011	−.195	−.108
	Plots	−.334	−.365	−.334	−.365
1930	Nursery	−.438	−.102	[a]−.499	[a]−.134
	Plots	−.148	.200	−.139	.207
Average, nursery		−.174	−.028	−.294	−.140
Average plots		−.301	−.190	−.316	−.245

PROTEIN-ABSORPTION

Year	Nursery or plot	Zero order	Loaf volume	Loaf weight	Volume and weight
1927	Nursery	0.077	0.092	0.219	0.167
	Plots	.078	.067	.198	.352
1928	Nursery	[a].412	[a].344	[a].481	[a].381
	Plots	−.056	−.027	−.007	−.009
1929	Nursery	[a].198	.223	[a].257	.246
	Plots	−.138	−.018	−.138	−.018
1930	Nursery	.271	.254	.363	.281
	Plots	.273	.244	.269	.250
Average, nursery		.253	.239	.321	.263
Average, plots		.027	.056	.056	.120

TABLE 7.—*Total and partial correlation coefficients and weighted averages secured from two diverse series of wheats, from the nursery and the plots, calculated from the four characters shown, 1027–1939*—Continued

ABSORPTION-WEIGHT

Year	Nursery or plot	Zero order	Protein content	Loaf volume	Protein and volume
1927	Nursery	0.673	0.690	0.682	0.690
	Plots	.617	.634	.618	.676
1928	Nursery	[a].422	[a].489	[a].552	[a].571
	Plots	.103	.087	.088	.085
1929	Nursery	[a].382	[a].412	[a].387	[a].399
	Plots	.022	−.026	.004	−.003
1930	Nursery	.122	[a].278	.283	[a].307
	Plots	−.053	−.013	−.002	−.054
Average, nursery		.437	.494	.499	.514
Average, plots		.162	.157	.164	.168

ABSORPTION-VOLUME

Year	Nursery or plot	Zero order	Protein content	Loaf weight	Protein and weight
1927	Nursery	−0.005	−0.052	0.152	[a]0.051
	Plots	.042	−.015	−.065	−.303
1928	Nursery	[a].298	.180	[a].479	[a].379
	Plots	−.053	−.018	.000	.006
1929	Nursery	−.040	−.111	.077	−.002
	Plots	−.185	−.126	−.184	−.123
1930	Nursery	.112	−.058	.279	.160
	Plots	.172	−.118	.165	−.129
Average, nursery		.076	−.021	.233	.133
Average, plots		−.026	−.074	−.034	−.127

VOLUME-WEIGHT

Year	Nursery or plot	Zero order	Protein content	Water absorption	Protein and water absorption
1927	Nursery	−0.172	[a]−0.127	[a]−0.227	[a]−0.127
	Plots	.151	.326	.159	.434
1928	Nursery	−.282	−.280	−.471	−.429
	Plots	−.516	−.275	−.514	−.275
1929	Nursery	−.283	[a]−.265	−.290	[a]−.243
	Plots	−.097	.183	−.095	.182
1930	Nursery	[a]−.700	−.531	[a]−.724	[a]−.618
	Plots	−.293	−.320	−.288	−.324
Average, nursery		−.362	−.293	−.426	−.344
Average, plots		−.219	−.027	−.216	−.004

[a] These pairs show significant differences. Any values of the weighted means numerically larger than 0.130 may be considered to show a significant difference.
[b] Total.

The coefficients in Table 7 are not accompanied by their probable errors. In lieu of giving the probable errors, 5 P values for $n=45$, 50, 60, 70, and 80 from Table V. A. of Fisher (*4*) are shown in Table 8.

TABLE 8.—*Correlation coefficient values for selected levels of significance (from Fisher) for coefficients shown in Table 7*

	$P=0.1$	$P=0.05$	$P=0.02$	$P=0.01$
45	0.243	0.288	0.338	0.372
50	.231	.273	.322	.354
60	.211	.250	.295	.325
70	.195	.232	.274	.302
80	.183	.217	.257	.283

In using these P values it must be remembered that n represents the net number of variates after deduction has been made from n' (= total variates) to secure the proper number of degrees of freedom. For the total correlation $n = n' - 2$ and with each variable held constant, an additional unit is taken from n', this last-named value equaling the total variates involved. Simple interpolation can be used for in-between values of n. Coefficients with values larger than those indicated naturally have P values less than those shown and are of greater significance. The pairs of correlations that have differences with P values of 0.05 or less (Table 7, footnote *a*) show significant differences.

PROTEIN-VOLUME COEFFICIENTS

Most of the zero-order protein-volume coefficients are fairly high. The correlation is uniformly higher for the plots, and in three out of four cases the difference is large enough to be significant.

Coefficients of correlation previously reported in the literature for the protein-volume relation have been summarized in Table 1. The average of the nursery for four years is 0.441, which is somewhat higher, but comparable to, the coefficients summarized in Table 1. The average of the plots, however, is 0.735, which is considerably higher than other coefficients reported, with the exception of the value 0.670 reported by Harris (*5*).

Coefficients of correlation which have been calculated from diverse strains of varieties and without regard to crop year probably do not represent as sharp reactions between protein and loaf volume as one finds in commercial mills where one wheat variety from one crop very often dominates the blend of a mill mix.

If the variety factor is held constant, therefore, as in the plots, there is a relatively high degree of correlation, averaging 0.735, between protein and loaf volume. This correlation for the plots would be sufficiently high to justify the payment of substantial premiums for protein content. The question arises in this connection whether these samples have been subjected to as much diversity as would a series of samples taken from a commercial mill. In the present instance the run of the weather for parts of four years is represented, rather than that from geographical weather variations of a single year, which one would look for in a commercial lot. In a commercial lot, also, a diversity of soil types and an appreciable amount of varietal difference would affect the degree of correlation between protein and loaf volume.

Not only are the zero-order protein-volume coefficients of the plots decidedly larger than those from the nursery, but their value is maintained better when the other two components, weight and water absorption, are held constant, either singly or together. In case of samples from the nursery, weight and water absorption contribute a significantly larger amount to the total correlation than do the samples from the plots. In the latter case also, the average decrease in coefficient values for the 12 possible comparisons is but 0.008 in comparison with the corresponding average of 0.060 for the nursery correlation. The difference for these two values is significant. When weight and water absorption are held constant, the 4-year average change from the total correlation for the plots is 0.008 as compared with 0.116 for the nursery.

When a single variety is grown under diverse conditions, the total protein-volume correlations are less affected, in this comparison, by holding weight and absorption constant than when several varieties of wheat are grown under the same conditions. This is merely a suggestive conclusion, as there are too few comparisons for significance.

VARIATION IN WHEAT PROTEIN-VOLUME AND FLOUR PROTEIN-VOLUME CORRELATION

Zinn (*13*) found high correlations between protein of wheat and protein of flour. Treloar and others (*12*) determined the correlation coefficient between the protein in the flour and in the wheat and found a weighted average value of 0.948 for a period of six years. The correlation coefficients between these two characters and loaf volume ran closely parallel for the various years, the two weighted averages not showing a significant difference. In such cases, it would evidently matter but little whether the correlation dealing with loaf volume was concerned with the wheat or with the flour protein. Since the flour used in this case was a comparatively short patent (75 per cent) it seemed advisable to determine correlations in regard to this point. Wheat-protein data were lacking for the nursery samples for 1927, and results are presented for three years only in Table 9.

TABLE 9.—*Correlation coefficients between protein in wheat and protein in flour and the correlation of each of these with loaf volume for the samples from the nursery and the plots, 1928–1930* [a]

Characters correlated	Correlation coefficients in—					
	1928		1929		1930	
	Nursery	Plots	Nursery	Plots	Nursery	Plots
Protein in wheat and in flour	0.953	0.978	0.827	0.980	0.807	0.988
Protein in wheat and loaf volume	.324	.777	.330	.648	.473	.854
Protein in flour and loaf volume	.351	.712	.322	.674	.583	.850

[a] The significance of these correlation values can be obtained from a reference to Table 8.

The correlations between protein in wheat and protein in flour are lowest in all cases for the nursery samples. Differences are strongly significant for 1929 and 1930. It seems rather likely that correlations between the protein content of wheat and flour are greater where only one variety is concerned, even though grown under diverse conditions, than where several varieties are involved, even when these are grown uniformly.

For 1930 the correlation between wheat protein and loaf volume is appreciably lower than that between flour protein and loaf volume for the nursery plots. In 1929, however, the correlation for the nursery samples between wheat protein and volume is slightly higher than that between flour protein and volume.

In 1928 a lower correlation was found between flour protein and loaf volume for the plots, but the reverse was true in 1929. From the foregoing, one can not conclude that there is any great advantage in using the flour-protein figure as compared with wheat protein even though the correlation between the two protein contents is not extremely high in some cases.

CORRELATION OF PROTEIN TO WEIGHT

The zero-order correlations are negative in all cases, but only in three of eight cases are the coefficients significant. The changes, from the total to the partial coefficients when volume or absorption, or both, are held constant, are of no particular moment. Generally there is a slight tendency for loaf weight to be depressed with an increase of protein. For the plots, 1928, a coefficient of -0.488 is shown. When loaf volume, or loaf volume and water absorption are held constant in this case, the coefficient is reduced to -0.200 and -0.199, respectively. The plot samples for 1928 contained sprouted wheat which likely tended to increase volume, on account of increased rate of fermentation, with a consequent tendency to lower the loaf weight.

CORRELATION OF PROTEIN TO ABSORPTION

The correlation coefficients between protein and absorption are not striking, although the majority are positive. The changes from the total to the net correlations are unimportant. The correlations for the nursery samples are positive throughout, while for the plot samples there are two positive and two negative coefficients. This would indicate that the relation between these two characters is more consistent when the series is diverse as to variety and genetic origin. The variation between different crop years is quite large.

Correlation results for the three characters volume, weight, and water absorption, in relation to protein, show that striking results are found only in the protein-volume relationship. Loaf weight and water absorption evidently are not closely related to protein content, and other factors apparently have greater influence on these two characters than has protein content.

CORRELATION OF WATER ABSORPTION TO WEIGHT AND VOLUME

Considering the relationship of water absorption to loaf weight, diverse results are encountered, and only in 1927 does one find comparatively high total correlations. For the nursery, all four of the zero-order coefficients are positive, and three are significant. For the plots, three coefficients are positive and one negative, but only one coefficient is significant. Mangels (*10*) reported coefficients of correlation for absorption and weight for four crop years the average of which was 0.730. Mangels used a series of wheats of different varieties from crops of 1923 to 1926, inclusive. The corresponding coefficients from the nursery samples for four years averages 0.437, which is significantly lower. This difference may perhaps be ascribed to seasonal variation. The partial correlations do not show appreciable differences from the zero-order coefficients.

The absorption-volume correlations are of comparatively little importance since in only a few instances do they become significant. These results are similar to those obtained by Mangels (*10*) for crops of 1923 to 1926, except that the coefficients reported by Mangels are positive in all cases. Comparing the zero-order coefficients for the nursery samples and for the plots, both series show two negative and two positive coefficients. When protein and weight are held constant, however, the nursery data show three positive and one negative coefficient for the four years, while the plots show three negative and one positive coefficients.

CORRELATIONS OF VOLUME AND WEIGHT

The correlations under this heading are of greater interest, generally, than those of the four preceding groups. Evidently not much attention has been paid to this phase of correlation, for no results seem to be reported in the literature. Only 6 of the 32 coefficients are positive, and but 2 of these are significant. Eighteen of the 26 negative coefficients are significant. It is evident that the relationship between volume and weight is not directly operative or organic. If the loaf weight tends to be depressed with an increase in size, then this relationship must be referred to protein or to absorption, or to both, in this present study. Considering this particular negative correlation the question of linearity immediately arises; there is evidently a limit to the continuous separation of these two characters. By taking the total correlation for the 1930 nursery samples, which lies at -0.7, a table was made showing the distribution of the variates. This is shown in Figure 1. Within the limits of the indicated class values the evidence for nonlinearity is not apparent. Fisher's test (*4, p. 219*) was applied, and the difference z, indicating lack of linearity, was wholly inappreciable. With this result the writers calculated the partial coefficients, which would have been a questionable procedure with much curvilinear regression.

Weight of loaf (grams)

Volume of loaf (cubic centimeters)	126	127	128	129	130	131	132	133	134	135	136	137	
385							1				1		2
395							1	1					2
405										1	2	1	4
415						1				1			2
425							1	1		1	2		5
435								1	2	1			4
445					2		2	2					6
455					1					1			2
465				1	2							1	5
475									1				5
485						3	1	1					4
495			1			1	2						2
505				1			1						4
515		1	1		1	1							3
525				1		1	1						1
535	1												1
545	1												2
555		1		1									2
565		1				1							1
575			1			1							1
	2	3	3	4	6	9	10	6	3	5	5	2	58

FIGURE 1.—Correlation diagram showing relationship between volume and weight of loaf for 58 variates from the 1930 nursery samples. The regression is linear and the correlation is -0.7

With the negative correlation between volume and weight it may be inferred that in those loaves in which the diastatic activity was greatest, with a resultant large gas production, a comparatively large loss of weight automatically took place. This loss in weight is presumed to be due largely to the destruction of carbohydrates by the yeast. In studying the volume-weight correlations one is struck by their lack of uniformity, either with regard to conditions of growth or to the individual year. When water absorption is held constant the changes from the total correlation are only slight. When protein is held constant, the changes from the total to the partial correlation are mainly positive and are distinctly significant.

Considering the correlation relations of the six groups of Table 7 only three, those concerned with protein and volume, absorption and weight, and volume and weight, have coefficients sufficiently large to make the correlations important. The correlations of the protein-volume group are the largest and most uniform. In general, the changes in size of coefficients, from the zero order to the partial, are not uniform within a group and are not of much importance.

Interesting as the foregoing results may be, it should not be forgotten that the correlation values in themselves do not exhibit the full measure of their importance. It is necessary to consider the regression coefficients, calculated from the standard deviation and the coefficients of correlation. From the regression coefficients it is possible to estimate deviations in loaf volume, for instance, according to any arbitrary deviation in protein.

REGRESSION COEFFICIENTS

While the total regression coefficients are worked out very simply according to the formula $b = r_{12}\frac{\sigma_1}{\sigma_2}$, the partial regression coefficients are calculated by a set of normal equations. An outline of the method is given by Fisher (*4, p. 133–135*). In calculating these, attention was paid mainly to the regression of volume on the two characters, protein and loaf weight. The values for absorption were usually not significant. The total and partial regressions expressed in cubic centimeters of loaf are shown in Table 10.

TABLE 10.—*Total regression coefficients of loaf volume on protein and on loaf weight and partial regression coefficients of volume on protein and on weight, with respect to absorption and weight, and to absorption and protein*

[Values are expressed in cubic centimeters of loaf]

Year	Nursery or plot	Protein		Weight	
		Total	Partial	Total	Partial
1927	Nursery	*18.42	*17.35	−1.81	−1.62
	Plots	*14.12	*14.99	.90	*1.60
1928	Nursery	*19.53	8.30	*− 5.83	*−9.37
	Plots	*39.51	*33.36	*−14.12	*−6.33
1929	Nursery	*13.59	*12.53	*−4.77	*−4.24
	Plots	*11.81	*12.42	−.90	1.31
1930	Nursery	*36.65	*18.17	*−11.88	*−10.11
	Plots	*19.24	*19.07	*−6.68	*−3.91

* These values are distinctly to highly significant. The other values are not significant, or scarcely so.

The values in Table 10 are to be interpreted as loaf-volume deviations in cubic centimeters for every respective unit deviation in protein or weight. The total regressions are calculated with regard to only two characters, whereas the partial regressions take account of all four characters, The difference between the total and the partial correlations is only slight in some cases but very considerable in others. Where one finds pronounced differences the partial coefficient is usually the smaller. A comparison between these coefficients and the correlation values of Table 7, made by averaging the total and second-order correlations and the total and partial regression coefficients for the four years, is shown in Table 11.

TABLE 11.—*Weighted averages of correlation and regression coefficients taken from Tables 7 and 10 for samples from nursery and from plots for the two relationships: Protein-volume and weight-volume*

Characters correlated	Total				Partial			
	Correlation		Regression		Correlation		Regression	
	Nursery	Plots	Nursery	Plots	Nursery	Plots	Nursery	Plots
Protein-volume	0.441	0.735	21.41	25.60	0.337	0.726	14.15	20.07
Weight-volume	−.362	−.219	−5.60	−3.77	−.344	−.004	−5.86	−1.87

Table 11 shows briefly what has been indicated previously. Under "total" for protein-volume, the two regressions differ by about 4 units, but the two correlations differ rather markedly. Under "partial" the two correlations differ still more, the regression average from the plots being nearly 6 units more. For weight-volume the nursery correlations are appreciably the larger in both cases; the partial regression values show more difference than is found between the total regression values. In brief, the plot samples show a direct relationship when volume is considered with protein, but with regard to the relationship of weight to volume the nursery samples show the higher values, either positive or negative.

It has been suggested to the writers by Doctor Treloar of the University of Minnesota that it is possible to combine into one correlation table paired data extending over a series of years (or events) with a correlation coefficient equal to the weighted mean coefficient secured by averaging the coefficients of the individual years. In order to do this it is necessary to eliminate the disorderly differentiation found from season to season due to differences in means and the corresponding disorderliness due to the various series of deviations from the different means. This is done by securing ratios of each series of deviations to the corresponding standard deviations. All standard deviations are thus reduced to unity. This was done with the plot and nursery samples for the four years with regard to protein and loaf volume. The two results, for the relationship of protein and volume, are as follows:

Nursery, 1927–1930	0.439
Plots, 1927–1930	.711

The results are shown in Figures 2 and 3. As the standard deviation values are unity, the correlation coefficients become the regression

values. In considering values like the one just presented, their limitations must be strictly considered. They can not be used for prediction purposes but may be used in a comparison of two or more sets of data combined into one set, with a similar combination. In a comparison of this sort differences may be observed in the slopes of the two lines present and also the disposition of the means of the arrays of each of the two sets. Considering the two correlation surfaces, one can compare the disposition of the two groups of variates. In Figures 2 and 3 the slope of the regression line is greater in Figure 3, the approximation of the means of the arrays to the regression line is greater in this figure, and finally the compactness of the variate groups shown in Figure 3 is greater. The ratio of the two slopes is 1.6. It will be recalled that the variability of protein was appreciably greater for the samples from the plots than for the nursery samples. This confirms what has already been indicated, that a series of protein values have greater influences on the loaf volume when they are restricted as to genetic origin, although unrestricted as to cultural conditions. Comparatively high variability in the protein content need not operate against high prediction values with respect to the volume of the loaf.

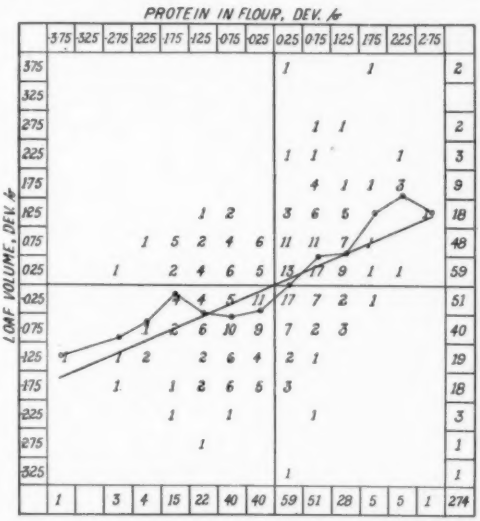

FIGURE 2.—Correlation diagram showing relationship between loaf volume and protein content of wheat from the nursery for 1927 to 1930. Differentiation of the means and standard deviations from year to year are eliminated by using the ratios indicated. The sloping line is the regression of the loaf volume on protein content expressed in ratios and is equal to the weighted mean of the four annual coefficients of correlation, which is equal to 0.439. The standard deviations are each equal to unity

APPLICATION OF REGRESSION COEFFICIENTS

A clearer idea is gained of the meaning and application of the regression coefficients if they are applied directly to the values concerned. In Table 12 the various regression formulas are shown. In addition the various expected loaf volumes are calculated, assuming the flour protein content to be uniformly 12 per cent. This protein percentage for flour is higher than that generally found in commercial flours but is one-half per cent lower than the average of the flours dealt with here. The ranges of loaf volumes are shown within which 80 per cent of the loaves are expected to fall according to a normal distribution. The theoretic ranges are compared with the actual, and the number of loaves coming under, and also over, the theoretic limits are indicated.

TABLE 12.—*Regression formulas derived from total regression coefficients with ranges of loaf volume expected to include 80 per cent of the variates, assuming a protein content of 12 per cent for each year, and the number of variates falling below and above the theoretic limits*

Year	Nursery or plot	Formula (volume=)	Number of variates	Mean		Most likely volume at 12 per cent protein	Range within which 80 per cent of variates are expected	Actual protein content minus assumed value	Variates outside theoretic limits	
				Protein	Volume				Under	Over
1927	Nursery	18.42 protein + 173.4	76	13.1	415	394	359–429	+1.1	0	20
	Plots	14.12 protein + 226.4	51	10.4	373	396	379–413	−1.6	34	0
1923	Nursery	19.53 protein + 164.9	59	14.8	454	399	328–470	+2.8	3	34
	Plots	39.51 protein + 1.0	70	12.2	484	477	400–554	+.2	1	10
1929	Nursery	13.59 protein + 263.3	81	10.8	410	426	376–476	−1.2	13	3
	Plots	11.81 protein + 272.4	80	12.1	415	414	391–437	+.1	5	7
1930	Nursery	36.65 protein − 25.5	58	13.4	466	414	353–475	+1.4	0	22
	Plots	19.24 protein + 211.9	58	13.1	464	443	410–476	+1.1	0	17
1923	Nursery	[a]8.30 protein + 331.1	59	14.8	454	431	360–502	+2.8	5	7
1930	do	[a]18.17 protein + 222.1	58	13.4	466	440	379–501	+1.4	0	30

[a] As above with 2 partial regression formulas derived from partial regression coefficients.

Table 12 shows that the number of loaves under and over the theoretic limits is anything but uniform. Obviously this is due to the rather wide deviations in the protein content of the flours. As the assumed 12 per cent protein content is below the average of 12.5 per cent, the number of loaves over the maximum limit might be expected to be larger than those under the minimum limit and this is actually the case, the difference being 57. The number of loaves under and over the limits of 80 per cent is considerably in excess of the theoretical 20 per cent, and this is also due to the arbitrary placing of the protein per cent.

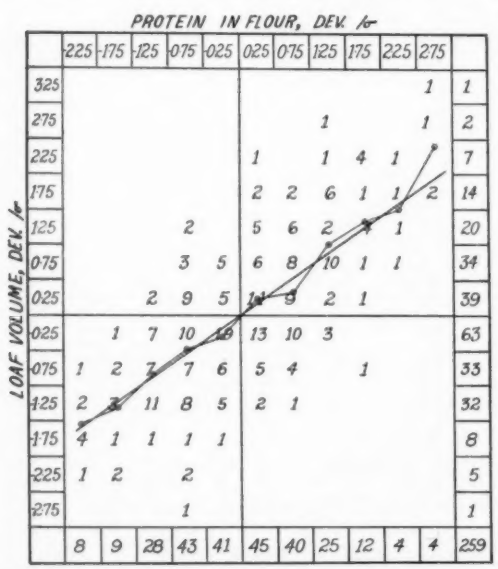

FIGURE 3.—Correlation diagram showing relationship between loaf volume and protein content of wheat from the plots for 1927 to 1930. Differentiation of the means and standard deviations from year to year are eliminated by using the ratios indicated. The sloping line is the regression of the loaf volume on protein content expressed in ratios and is equal to the weighted mean of the four annual coefficients of correlation, which is equal to 0.711. The standard deviations are each equal to unity

The partial regression formulas were also calculated; and as they gave nearly identical expected loaf volumes (with two exceptions), the results are not presented, except the two referred to, which are shown at the bottom of Table 12. The number of loaves outside the lower and upper limits are more equalized for the 1928 nursery sam-

ples, using the partial regression coefficients, but are less uniform for the 1930 nursery samples. The various limits within which 80 per cent of the variates are presumed to fall are in direct proportion to the respective standard deviations of loaf volume. This is evident from the fact that the latter are multiplied by a constant abscissal value x distant ± 1.28 from the central ordinate of the normal curve.

In order to make a comparison of prediction values of the two series, weighted means were taken of both the total and partial regression equations. This appears to be a legitimate procedure, as these equations contain essentially only means and correlation coefficients, which are both subject to weighted means. In taking the weighted means of the standard deviations the variances rather than the standard deviations were weighted. The following equations were thus obtained:

Weighted-mean total-regression equations

Nursery: Volume = 21.41 protein + 162.3
Plots: Volume = 25.60 protien + 128.7

Weighted-mean partial-regression equations

Nursery: Volume = 14.15 protein + 251.5
Plots: Volume = 20.07 protein + 193.2

When the two pairs of regression equations are calculated, the differences in the coefficient portions are nearly the same. The protein coefficient for the plots is larger in each case and probably significantly so. The difference between the coefficients is only slightly larger in the case of the partial correlations. These differences, though perhaps not of great importance, show the protein of the one variety, grown on the plots, to be the more potent in its effect on loaf volume. It is obvious that regression lines, calculable from the equations shown above, would scarcely be applicable to a correlation surface. As the correlation surfaces shown in Figures 2 and 3 deal with ratios and not with actual values, the regression lines from the above equations, which deal with actual values, could not be drawn on the surface nor used for prediction purposes in connection with ratios. If the paired values between protein and volume for the four years of a series were to be entered on a correlation surface the "disorderly differentiation," appearing when seasonal results are compared, discussed by Treloar and others (*12*), would dominate the situation and would not accord with the weighted regression lines. In such a case, also, the individual years would not be handled as units.

DIVERSE SEASONAL EFFECTS

Seasonal conditions have a marked influence, as is well known, on the amount and quality of protein. Table 2 shows that the amount of protein from the plot samples is essentially the same for the two years 1928 and 1929. Loaf volume, on the contrary, showed nearly three times as much variability for 1928 as for 1929, a relatively enormous difference. The correlation coefficients for the two years, however, did not show a significant difference. These facts, along with the regression equations, are shown in Table 13.

TABLE 13.—*Means of protein, variabilities of loaf volume, coefficients of correlations, and regression equations to show seasonal diversity of protein effect in two successive years, shown by plot samples*

Year	Protein	Variability of volume	Coefficients of correlation, (P–V)	Regression equation (volume=)	Volume deviation in cubic centimeters per units of protein
	Per cent	*Per cent*			
1928	12.2	12.43	0.712	39.51 protein+1	40
1929	12.1	4.28	.674	11.81 protein+272	12

The prediction value of the protein as to loaf volume is more than three times greater for 1928. The foregoing clearly indicates that neither the amount of protein nor the degree of correlation between protein and loaf volume is always a dependable criterion as to the market value of protein. This remarkably different behavior of the protein relative to loaf volume that may occur from a single variety in two different seasons grown upon a group of plots with diverse manurial and crop sequence treatments is presented in Figure 4. This is a scatter diagram of loaf volume and flour protein for the two years with the two regression lines superimposed.

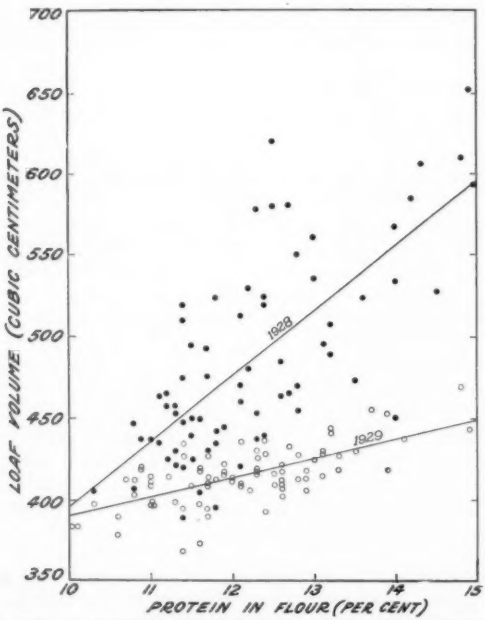

FIGURE 4.—Scatter diagram showing relationship between loaf volume and protein content of flour for the wheat samples from the plots for two successive years. The regression coefficients for 1928 and 1929 are 39.5 and 11.8, respectively

DISCUSSION

In the production of a loaf of bread there is involved a highly complicated set of physical and chemical reactions. The basic material, flour, is a colloid mixture in a dry condition, necessarily complicated, to which is added other substances, water, minerals, yeast, fat, and sugar, previous to subjection to heat, to produce the finished loaf. An immense number of loaves must be made, and these must be made from flours ground from various grades and classes of wheats. A desirable loaf must fall within rather narrow limits to satisfy the public taste and fashion.

As a consequence the millers and bakers seek for a single criterion, or for only a very few criteria, to apply to the flour in evaluating the future loaf. Because of its unique properties, protein in wheat flour has received a great deal of study. This substance is considered so important that variations in wheat prices are based on the amount of protein in many cases. This being the case, it is evident that a very full knowledge of the protein content of the wheat and the resulting flour is demanded. This knowledge is of a twofold nature: On the one hand, a study is needed of the relationships affecting the laying down of the protein in the wheat kernel during the formative period, with regard to variety and the total environment, and on the other hand a study is needed of the relationships of the protein, as laid down, to the loaf in its various stages. While considerable knowledge of this sort is already available, a satisfactory goal has not been reached.

In the present paper, data are presented from two series of hard red spring wheats which throw some additional light upon the protein problem. The nursery series, grown under comparable conditions, varied genetically, while the plots series, grown under varying conditions, consisted of samples genetically uniform. The higher-average protein content shown by the nursery samples, as an average for the four years, may be ascribed very likely to sweetclover turned under, preceding the wheat crop by one or two years. In 1929 when the protein content for the nursery samples was lower than that of samples from the plots, oats preceding the nursery wheat evidently caused the low protein content. Evidence is presented to show the much greater influence exerted on the protein content by soil variability, developed in the rotation and fertility plots, than by genetic differences present in the nursery samples. Evidence for this is very positive for three of the four years but neutral for the fourth year.

If the two series of wheats are compared as two units and not by years, the variability of the protein from the plots is found to be much the greater. The coefficients of variability for the two series are: Plots 18.43 ± 0.56, and nursery 12.56 ± 0.37. The difference is 5.87 ± 0.67 which is highly significant. This is a different sort of a comparison, however, than when the comparative variability is considered year by year.

Variability of the protein content of a series of wheat samples is apparently due more to the result of external conditions, soil and climate, than to the genetic character of the wheat. Put in another way, a series of protein values will show greater variability when affected by a random sampling of external conditions than when affected by a random sampling of internal (genetic) conditions.

A discussion of climate in the present paper has been almost entirely omitted as it was believed that a consideration of that factor would complicate the discussion more than was desirable. It is not believed that weather conditions differed enough to affect the comparisons. Either of the two series, and especially the plot series, affords excellent material to study the effect of weather on the amount and character of protein.

With the characters of water absorption, loaf volume, and loaf weight, the effect is generally contrary to the finding for protein, for in these cases genetic differences are responsible for a strikingly greater degree of variability than are the environmental conditions, diverse

as to soil fertility and crop sequence. It is fair to presume that a similar statement would hold if a comparison were made between genetic differences and climatic environmental conditions. It is evident from this that although the amount of protein may show marked variability, this variability need not be much reflected in the variability of loaf characters.

Correlation data were calculated between the four characters, protein, water absorption, loaf volume, and loaf weight, for the samples from the nursery and from the plots for the four years under consideration. The correlation coefficients showing the relationships between protein and volume are uniformly higher for the samples from the plots where one variety was grown than from corresponding samples from the nursery, which were genetically diverse. Evidently where diverse strains or varieties enter into the correlation study, one does not find so sharp reactions between protein and loaf volume as are found in commercial mills where the flour output consists of, or is dominated by, one variety. The correlations between protein and volume were distinctly higher than between any other pair. Correlations were positive and significant in all cases. In the five remaining cases the coefficients were only occasionally significant for the samples from the nursery or from the plots for the four years. For two series of correlations, those showing relationship between protein and weight and between volume and weight, the coeffcients were almost uniformly minus, and in a few cases rather distinctly significant. Evidently fermentative activities which tend to produce large volumes do so at the expense of some of the carbohydrate material in the loaf. In one instance where the coefficient between volume and weight was -0.70 the regression was essentially linear for the class limits which existed. In this extreme case, where the loaf showed the greatest loss in weight, associated with large volume, it was found that the weight of the loaf suffered about 1.5 per cent, using the standard deviation of loaf volume as the criterion.

The net or partial correlation coefficients were calculated. In most of the cases the change from the total to the net correlations is not marked. In only a few cases was the change significant. In the other relationships the total correlation coefficients were generally low, and no marked change in absolute values would be expected in comparing total and net coefficients.

It is not evident from the data that there is any great advantage in using the flour protein in obtaining the correlations even though correlations between wheat protein and flour protein are not extremely high. Treloar and others (*12*) held wheat protein and flour protein alternately constant and correlated for the other character. In doing this it became evident that the wheat protein was a better criterion of loaf volume than the flour protein. They conclude that those wheats of the hard red spring class which have a higher protein concentration in the bran than in the endosperm are of somewhat better baking quality relative to loaf volume. In the present study, with only three years' data available, higher correlations between wheat protein and flour protein seem to exist where only a single variety is concerned rather than with many varieties.

In calculating the total and partial regression coefficients of volume on protein, significant amounts were found in practically all cases. In fact, the value of this relationship was rather dominating relative

to other relationships. Figure 4 shows the marked difference there may be as to the effect of protein on the loaf from one season to another, irrespective of the quantity of the protein and with but little regard to the degree of correlation. In following out the idea of Treloar by eliminating the disorderliness appearing in the data from season to season, correlation surfaces are obtained easily comparable with one another and whose "regression lines" are equal to the respective weighted correlation coefficients. Although the ratio of the two slopes of the nursery samples to the plot samples was 1.6, this does not express the relative difference in the prediction value of the protein to loaf volume. When the two weighted regression coefficients are calculated, for the nursery and for the plots, the difference between them is relatively low, in contrast to the rather marked difference between the two correlation coefficients. This evidently arises from the facts already emphasized—that while the variability of the protein from the plots is relatively high, the variability of the loaf volume (and also water absorption and loaf weight) is relatively low.

SUMMARY

Two series of hard red spring wheats are compared in regard to the means, coefficient of variability, correlations (total and net), and regression equations relative to the character of protein content, water absorption of flour, loaf volume, and loaf weight. One series, designated as nursery, consisted of wheats diverse in genetic origin but grown under uniform conditions of soil and soil treatment, while the other consisted of a single variety grown on plots that were subjected to different treatment in regard to crop sequence and soil fertilization.

The flour-protein content of the nursery samples was higher than for the plot samples for the four years, which was evidently due to the fact that sweetclover had been plowed under previous to the wheat. The unweighted means of water absorption, loaf weight, and loaf volume are very close together; the small differences show no significances.

The protein content showed significantly greater variability for three years for the plots and a less, but nonsignificant, variability for one year. Considering the two series as units, the variability of the protein from the plots showed distinctly greater variability for the four years, the difference being 5.87 ± 0.67.

In contrast to the above, the nursery samples showed moderately to distinctly greater variability in 9 of the 12 cases where water absorption, loaf weight, and loaf volume were concerned. In only one instance was the variability greater for the plot samples.

Varied soil and crop-sequence conditions acting on a genetically uniform wheat resulted in greater protein variability than did genetically diverse wheats under uniform conditions. Genetic variability, on the other hand, expressed through uniform soil conditions, resulted in greater variability for water absorption, loaf weight, and loaf volume in comparison with a single variety grown diversely as to soil fertility and crop sequence.

Complete total and partial correlation coefficients were secured The protein-volume correlations were of greatest moment, and those from the plots were higher than from the nursery samples in all cases

and significantly so in 11 of the 16 comparisons. This leads to the conclusion that a more direct relationship exists between protein and loaf volume in a commercial mill, where the wheat of one variety very often dominates the blend in a mill mix, than in a genetically diverse group of wheats commonly grown experimentally.

Correlations of protein and water absorption and water absorption and volume were generally small and not significant. There was no significant relationship of loaf weight to absorption relative to the plot samples, but this relation generally was moderately significant so far as the nursery samples were concerned. Considering the relationships of protein and weight and volume and weight, the correlations were generally negative and of distinct significance in certain instances. In the extreme case, with a correlation of -0.7 between volume and weight, a loss of weight of 1.5 per cent would be expected as a result of baking. Within the class limits present, the foregoing negative relationship was linear.

In most cases the changes in value, from total to net correlations, were not of much importance.

For three years, where data were available, the correlations between protein in wheat and loaf volume and protein in flour and loaf volume showed no material differences.

Total and partial regression coefficients, with regard to regression of volume on protein, absorption, and weight, were calculated. The volume-absorption regressions were generally not significant. The volume-protein regressions were significant in practically all cases, both total and partial, and generally very distinctly so. The volume-weight regressions were negative in 13 out of the 16 cases, and 10 of the negative coefficients were significant. Seasonal effects showed markedly when the total regression equations were compared for two years from the plots. The loaf volume deviation in cubic centimeters for the deviation for each protein per cent was 40 for 1928 and 12 for 1929. Due to the variability in protein content from year to year, the annual range of loaves below and above the expected 80 per cent calculated from a uniform 12 per cent protein content was very unequal. The partial regression coefficients gave generally nearly the same prediction values for loaf volume based on protein as did the total regression formulas.

Correlation coefficients by themselves do not suffice to show to what extent loaf volume is dependent on protein content. Regression coefficients are necessary for a proper interpretation. In one comparison, with the two correlation coefficients nearly equal, a marked difference in loaf volume predictable from the protein was obtained from the results of two years. When correlation coefficients were significantly different, the regression coefficients at times approximated rather closely.

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SOME MINOR STAINS OF SOUTHERN PINE AND HARDWOOD LUMBER AND LOGS[1]

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INTRODUCTION

In the course of experimental work on the control of sapwood stain and mold in southern pine[3] and hardwood lumber and logs, various stains other than those of the ordinary blue-stain type were encountered frequently. Most of these ranged in color from bright yellow to deep red or purple, and under certain conditions were of sufficient intensity and prevalence to assume practical importance. In addition, certain common green surface molds were found to be capable of penetrating into pine lumber and producing a more or less shallow discoloration. Fresh pine sapwood stored under moist conditions was especially susceptible to stains and molds of this type. Such hardwoods as red gum,[4] yellow poplar,[5] and sweet bay,[6] were generally free from them; but cultural tests in the laboratory showed a few of the organisms involved to be capable of staining sap gum, at least to a slight degree.

These stains appeared to be associated with and possibly stimulated by certain chemical solutions with which the lumber and logs had been treated for the purpose of preventing blue stain. Thus, the yellow and red discolorations were common on wood that had been immersed in formaldehyde, silicofluoride solutions, benzoic acid and its salts, arsenious oxide, or boric acid; a blue-green mold was prevalent on lumber treated with ethyl mercury compounds; while several green superficial molds were commonly associated with the use of sodium bisulphite, sodium silicate, ammonium fluoride, formaldehyde, and arsenious oxide. Although such relationships between treatments and organisms seemed to exist, they were not specific, as each of the stains was later produced on fresh untreated pine sapwood under proper conditions of moisture.

Stains of a similar nature have been described by Hedgcock[7] in his study of chromogenic fungi that discolor wood. He found that certain Penicillia, principally *Penicillium aureum* Cda. and *P. roseum* Lk., commonly produce colors varying from yellow to crimson in the sapwood of pine and several hardwoods. In the case of *P. aureum*, the color was due to the secretion of a soluble pigment on the surface of the fungus filament. This pigment varied with the acidity or

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[1] Received for publication Jan. 20, 1932; issued September, 1932.

[2] For their kindness in identifying the organisms discussed herein, thanks are due Dr. C. D. Sherbakoff, of the Department of Botany, University of Tennessee, and Dr. Charles Thom, Principal Mycologist in Charge, Division of Soil Microbiology, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

[3] The southern pine lumber referred to in this paper was derived from the following species: Longleaf pine (*Pinus palustris* Miller), shortleaf pine (*P. echinata* Miller), and loblolly pine (*P. taeda* L.).

[4] The lumber known in the trade as red gum is derived from the sweetgum tree, *Liquidambar styraciflua* L.

[5] The lumber known in the trade as yellow poplar is derived from the tuliptree, *Liriodendron tulipifera* L.

[6] *Magnolia virginiana australis* Sargent.

[7] HEDGCOCK, G. C. STUDIES UPON SOME CHROMOGENIC FUNGI WHICH DISCOLOR WOOD. Missouri Bot. Gard. Ann. Rpt. 17: 59–114, illus. 1906.

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alkalinity of the culture media, being red when alkaline and yellow when acid, and was taken up by the cell walls of the wood. A third fungus, *Fusarium roseum* Lk., was identified as causing pink, red, and violet blotches upon fresh sapwood of *Pinus resinosa* Sol., *P. strobus* L., and several of the southern pine species. Coloration resulted from the secretion of a soluble pigment that was taken up by adjacent wood cells, staining them lightly red or purple, depending upon the acidity or alkalinity of the wood. Both Gerry [8] and Hedgcock [9] found that certain molds which usually occurred superficially on lumber were capable of shallow penetration and often produced mild interior discoloration.

In so far as could be determined, the fungi associated with the stains herein described, with the exception of one of the surface molds, are distinct from the species mentioned previously. It is probable that a considerable number of fungi are capable of producing less common stains of this nature under favorable conditions, although their distribution may be more or less limited regionally.

EXPERIMENTAL METHODS

A large number of isolations were made in both the field and the laboratory by gouging slivers from the surface and interior of discolored wood and transferring them to malt-agar slants. Those organisms that were isolated repeatedly and seemed involved in the staining of the wood were selected for the purpose of this study. Duplicate agar cultures of the Fusaria were sent to Doctor Sherbakoff and duplicate agar cultures of the other organisms to Doctor Thom for identification. To determine their staining ability and characteristics, each organism was planted upon culture blocks of pine and red gum sapwood. Previous to inoculation with the several fungi, the culture blocks had been submerged in boiling water for five minutes and transferred immediately to sterile 2-quart fruit jars plugged with cotton. After an incubation period of 50 days, reisolations were made from the resultant stains, and the gross cultural characteristics of the organisms on agar were compared with those of the original cultures. Field observations were correlated with cultural results wherever possible.

RESULTS

Under the almost ideal conditions for growth presented in the laboratory, all the organisms covered the blocks of both pine (*Pinus palustris* and *P. taeda*) and gum with a superficial growth within 7 to 10 days. Those capable of staining produced definite discoloration in the interior of the blocks within 30 days after inoculation. For convenience, the stains are grouped into four classes: (1) Purple-pink stains, (2) orange stain, (3) yellow stain, and (4) superficial mold stains. The organisms involved and some of their staining characteristics and effects on wood are summarized in Table 1.

[8] GERRY, E. FIVE MOLDS AND THEIR PENETRATION INTO WOOD. Jour. Agr. Research 26: 219-230, illus. 1923.

[9] HEDGCOCK, G. C. Op. cit.

TABLE 1.—*Effect of minor staining fungi on wood*

Stain and fungus	Wood from which fungus was isolated	Type of stain		Distribution of fungus in wood	Factors producing the stain
		In field	In culture blocks		
Purple-pink stains:					
Fusarium moniliforme	Sapwood of southern pine; red gum.	Purple and pink patches on pine; faint purple flecks on red gum.	Purple color throughout pine; small purple patches in red gum.	Confined largely to ray parenchyma and resin ducts of pine.	Colored hyphae and secretion of soluble pigment.
Fusarium solani	do	Purple patches, less intense on gum.	Similar to that of *F. moniliforme*, but less intense.	do	Do.
Fusarium viride?	Southern pine	do	Similar to that of *F. solani* on pine; less intense on gum.	Ray parenchyma and resin canals.	Do.
Orange stain:					
Unidentified fungus	do	Small shallow orange spots	Shallow orange spots	Vigorous surface growth; slight penetration into rays and resin ducts.	Do.
Yellow stain:					
Gymnoascuslike fungus	Southern pine; red gum	Yellow patches on pine; shallow spots on gum.	Yellow coloration throughout pine; shallow spots on gum.	Ray parenchyma, resin canals, and to some extent in tracheids. No bore holes evident.	Largely colored hyphæ and spores; soluble pigment to some extent.
Superficial stains:					
Penicillium expansum	Southern pine	Bluish-green surface discoloration; often general over piece.	Darkening of wood limited to immediate region of resin ducts.	Resin ducts principally; to some extent ray parenchyma and tracheids.	Compacting of hyphea and fruiting structures in resin ducts.
Gliocladium sp. and *Trichoderma* sp.	Southern pine; red gum	Green surface discoloration; often associated with blue-stain log infection.	do	do	Do.

The purple-pink stains caused by several species of Fusarium are of minor economic importance. They occur in small patches and do not involve the entire sapwood area, as the blue stain often does. They appear on lumber and logs in the early stages of drying and are prevalent only when moisture is unusually abundant. Under favorable conditions of moisture interior discoloration may be produced in lumber within 10 to 15 days after it is sawed. No reliable distinction can be made in the field between the several organisms involved, since the color differences exhibited on wood are slight, even in fresh cultures, and tend to fade as the wood dries. Moreover, differentiation is difficult in the laboratory because the several species vary considerably in color, form, and characteristics of growth, depending on the substratum upon which they are grown. Staining is accomplished through the presence of colored hyphae and the secretion of a soluble pigment which is absorbed by adjacent wood cells. Of the three species, *Fusarium moniliforme* Sheld. was isolated most frequently and produced the most intense discoloration both in the field and in the laboratory. *F. solani* (Mart.) Sacc. was often associated with purplish discolored areas in stored red gum logs, and is possibly the principal cause of such stains. The third species, provisionally identified as *F. viride* (L.) Wr., produced a heavy superficial growth of mycelium on culture blocks, but proved less capable of staining the wood than either of the other organisms.

The orange stain is produced in pine lumber and culture blocks by a fungus which could not be identified because of its failure to fruit. On Difco malt agar the mycelium is cream to buff-orange and semiappressed; it secretes a pigment that gives the agar a color varying from orange to coffee. This stain was found infrequently in the field and rarely assumed dimensions greater than small shallow spots or patches. It is probable that in many instances it was overlooked because of its inconspicuousness.

Under certain conditions, the yellow stain is common on pine lumber and occurs to some extent on red gum also. This stain appears on lumber in the early stages of drying and is prevalent only when there is an exceptional quantity of moisture present. The yellow color, although bright at first, tends to fade as the wood dries. Numerous isolations from such stained areas yielded only one type of organism, which was associated frequently with Gliocladium or Trichoderma mold. This organism produces asci abundantly. These show the characteristic shape of certain species of Aspergillus, but there are no Aspergillus heads on the colony. According to Thom, the general character of the colony is that of Gymnoascus, corresponding closely in form to *Penicillium luteum* Zuk. and its allies, but having an entirely different form of ascospore. On Difco malt agar, the mycelium and spores are from the first a decided lemon yellow, and in older cultures the agar may be changed to a definite brown. Hedgcock [10] describes *Penicillium aureum* as creating a lemon-yellow stain in pine wood or in acid media. This organism was not obtained in any of the isolations made in this study.

Superficial mold stains of several different colors occur on moist surfaces of pine lumber and logs and to some extent on gum, within as short a period as 36 to 48 hours after they are sawed. The stains

[10] HEDGCOCK, G. C. Op. cit.

appear first as white superficial growths and later assume their characteristic colorations on the surface of the wood. In the case of pine, discoloration is limited to a darkening of the wood in the immediate region of the resin ducts where the fungus filaments and spores are compacted. Of these molds, *Penicillium expansum* (Lk.) Thom showed a definite preference for both pine and gum lumber that had been treated with organic mercury salts, such as ethyl mercury phosphate and ethyl mercury chloride. *Gliocladium* sp. and *Trichoderma* sp. were encountered wherever lumber was stored under moist conditions. They seemed especially prevalent on lumber cut from infected logs, where they were usually associated with areas of blue stain. Several dipping treatments for lumber, containing fluoride salts, sodium bisulphite, formaldehyde, or arsenious oxide, seemed to encourage the development of molds of this type. The treatments giving the most satisfactory control were those with sodium carbonate and bicarbonate, sodium dinitrophenolate, sodium orthophenyl phenolate, or lime sulphur. The genus Gliocladium is regarded by Hedgcock [11] as one of minor importance in the staining of lumber.

SUMMARY

A number of stains other than those of the ordinary blue-stain type were found on lumber and logs stored under exceptionally moist conditions. The staining capacity of the fungi that were isolated repeatedly from such discolored wood was determined by inoculating culture blocks of sap pine and gum. Most of them proved to be superficial molds, but several produced stains, including purple-pink, yellow, and orange, throughout the blocks. Most of these stains belonged to the Fusarium and Penicillium groups. The more common superficial molds were found to produce either uniform shallow discolorations or discolorations limited to the immediate region of the resin ducts. Among the most common genera represented were Penicillium, Trichoderma, and Gliocladium.

These stain fungi were somewhat similar to the blue-stain type in that the mycelium was concentrated in the resin ducts and parenchyma cells of the wood rays but occurred to a limited extent in the wood and ray tracheids. Coloration was due either to the color of the mycelium alone or to the combined color of the mycelium and a soluble pigment that was absorbed by the adjacent wood cells.

Certain chemical solutions with which the lumber had been treated in order to prevent stain appeared to stimulate the growth of some of these organisms.

[11] HEDGCOCK, G. C. Op. cit.

THE RELATION OF AGRONOMIC PRACTICE TO THE QUANTITY AND QUALITY OF THE OIL IN FLAXSEED [1]

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INTRODUCTION

Present-day problems in agronomy are as much concerned with factors that affect the quality as with those that affect the quantity of the various crop plants. It is not only essential carefully to survey existing varieties in order to determine the range of their variability in composition, but it is also of paramount importance to be able to predict the effect of different agronomic practices on the quality of the crop. Such knowledge is available for many of the commonly grown crop plants, but for flax information of this sort is rather limited. Since agronomic practices in flax production are more variable than for many other crops, it is desirable to know in greater detail the relation between cultural practices and the composition of the seed.

The present series of investigation with flax were made to obtain additional data on the rate of development of the seed and the formation of oil on soils of different productivity, and to study the influence of delayed planting on the yield and composition of the seed.

PREVIOUS INVESTIGATIONS

Several investigators have studied the influence of fertilizer applications on the oil content of oil-bearing seeds. The studies with flax (*6, 7, 11*)[3] have shown that applications of commercial fertilizers may alter the oil content of the seed and also influence the various constants of the extracted oil (*11*). Garner, Allard, and Foubert (*8*) have shown that the oil content of cottonseed from plots where complete fertilizers were used was greater than that of the seed from the check plot, but that heavy applications of nitrogen relative to phosphorus and potassium reduced the oil content of the seed. Stark (*13*) determined the oil content of soybeans under various soil treatments and found that applications of lime and organic matter tended to reduce the oil content of the seed, and that rock phosphates added with lime and organic matter still further reduced it. Potash applications increased the oil content of the seed.

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[3] Reference is made by number (italic) to Literature Cited, p. 255.

Bushey, Puhr, and Hume (*3*) have studied the influence of crop sequence on the oil content of flaxseed. They analyzed 10 samples representing 10 rotations and found a difference of only 2.1 per cent between the sample containing the least oil and that containing the most. The differences obtained were not considered to be significant.

Rabak (*12*) has made an extensive study to determine the influence of climate and soil on the percentage and quality of oil in flaxseed. From four varieties of flax grown in 1914 and 1915 at seven locations in the middle and far-western part of the United States a considerable variation in the oil content of the seed was obtained. Tobler (*14*) has summarized the work of Ivanow, who studied the percentage and iodine number of the oil from flaxseed secured from 28 experiment stations in the flax-producing countries of the world. The oil content of the seed was found to be dependent on the kind (variety) and was not strongly influenced by geographical location. The iodine number of the oil, however, was influenced by the region in which the seed was grown. Seed secured from northern latitudes gave a higher iodine number than seed produced in southern countries.

A summary of the data obtained by the investigators referred to indicates that flaxseed may, under certain conditions, show considerable variability in composition as a result of differences in soil fertility and climate. Only a relatively small number of investigations have been made on the various phases of oil formation in oil-bearing seeds. Most of the studies with flaxseed have been made with bulk samples secured by harvesting flax plants at various stages in their development. Since flax blooms over a period of two weeks or more, bolls of different ages are found on the same plant. The difficulty of determining the exact stage of development of the seed by this method of harvesting is quite evident. Ivanow (*10*) harvested flax at four stages of maturity to study the oil-forming process. In 1910 the seed from the first harvest one week after flowering contained 4.4 per cent oil, the seed from the second harvest on July 18, 13 days later, contained 11 per cent, the seed from the third harvest on August 3 contained 32.5 per cent, and the seed from the fourth harvest on August 25 contained 35 per cent. These data show an extremely rapid rate of oil formation between July 18 and August 3, when practically all of the oil was laid down in the seed. The seed secured from each harvest was analyzed to determine the percentage of sugars. As the seed matured the percentage of sugars diminished, which suggests their conversion to oils during the oil-forming process. From a study of the percentage of oleic and linoleic acids and the iodine number of the oil at each harvest, Ivanow concluded that the saturated fatty acids were formed first during the synthesis of oil and from them the unsaturated fatty acids were derived.

The analysis of flaxseed for oil content at four degrees of maturity made by Eyre and Fisher (*6*) gave results which agreed quite closely with those of Ivanow (*10*). Eyre and Fisher suggest that flax may be harvested before it is fully mature without a serious reduction in the yield of oil. This is a common practice where flax is grown as a fiber crop.

Bushey, Puhr, and Hume (*3*) harvested flax planted on June 10 at five stages of maturity. The seed from the first harvest on August 25 was very immature but contained 29.51 per cent oil. The seed from

the fourth harvest on September 21, which was fully matured, contained 36.84 per cent. The iodine number of the extracted oil was lower at each of the five harvest dates. The maximum value was 181.5 at the first harvest and the minmum was 161.0 at the last harvest.

Dillman (*5*) has measured the growth in volume and the formation of oil in developing flaxseed. The seed used for the study was of known age, obtained by tagging a large number of flowers and collecting the seed at frequent periods of their development. It is evident that a study made by this method gives more accurately the rate of development of the seed, since the material used for each of the harvests is of uniform and known age. The results of Dillman's studies made in 1926 and 1927 at University Farm, St. Paul, Minn., and at Mandan, N. Dak., show that the growth in volume of the seed, as determined by measurements of length, width, and thickness, is relatively rapid, reaching a maximum at 12 to 14 days after flowering. The growth of the seed, as determined by the daily increase in dry weight, continued for a period of 33 days and then remained constant to the end of the ripening period at 40 days after flowering. The most rapid formation of oil, based on percentage of oil in the dry seeds, began at about the seventh day after flowering and continued for a period of 15 to 18 days. After the maximum percentage of oil was reached there was little or no significant change up to full maturity. The maximum percentage of oil was reached somewhat before the maximum dry weight of the seed.

All the data reviewed indicate that oil formation in oil-bearing seeds takes place most rapidly midway between flowering and maturity. Consequently, a study made during this period should show most clearly the relation of environmental conditions to oil content of the seed.

Bushey, Puhr, and Hume (*3*), working with flax, and Garner, Allard, and Foubert (*8*), with soybeans, have shown that delayed planting has very little effect upon the oil content of the harvested seed. In each instance, plantings were made at 15-day intervals over a 2-month period beginning at the normal planting date for the crop used. Each of these studies was conducted for a single year. One variety of flax and four varieties of soybeans were included in the tests. Stoa [4] has furnished unpublished data on the oil content of flaxseed from several dates of planting. His studies, covering a 7-year period, show conclusively that delayed planting has only a very slight influence on the oil content of the seed.

MATERIALS AND METHODS

Two varieties of flax, Bison (Minnesota accession No. 199) and Diadem (Minnesota accession No. 197) were used to determine the relation of soil productivity to the rate of seed and boll development and oil formation. These varieties were selected on the basis of considerable agronomic data from varietal trials at University Farm, Minn., and at branch stations. Bison is characterized by a fairly tall growth habit, mid-season maturity, and medium large seeds, and it has yielded well in variety trial comparisons. Diadem is mid short in growth habit, later in maturity, has fairly large seeds, and has given

[4] Stoa, T. E. Unpublished data.

comparatively low yields in varietal trials. The two varieties are therefore quite different in agronomic characteristics.

The two varieties were grown in 1929 on plots represented by two different rotations and a continuous cropping system. The plot treatments were started in 1899 and have been in continuous operation since. The 5-year rotation is composed of wheat, clover and timothy hay, pasture, corn, and oats. Barnyard manure has been applied preceding the corn crop. The 3-year rotation includes wheat, clover and timothy, and corn. In the continuous wheat, 6 pounds of red clover was planted each year and the crop growth was plowed under in the fall. No manure or fertilizer has been applied to the crops on the 3-year rotation or to the continuous wheat. The rotation plots were arranged to grow each crop every year, so that yield comparisons are available for all three plots used throughout the entire period of cropping. The yields of wheat in bushels per acre for the 4-year period 1924–1927 are 22.6 for the 5-year rotation, 18.6 for the 3-year rotation, and 15.3 for the wheat continuous. These data show considerable difference in the yield of wheat under the three different cropping systems.

The two varieties were planted on May 1 with a field grain drill on a portion of each of the three plots usually planted to wheat. Shortly before full bloom enough flowers were marked to supply seed for daily measurements and oil determinations. The flowers were marked by tying with a loose knot a small piece of colored woolen yarn just below the flower. Since it was impossible to tag on the same day all the flowers needed from each of the plots, a different colored yarn was used for each tagging date. Twelve thousand to thirteen thousand flowers were marked on each variety for each of the three plots, making a total of 75,000 flowers used in the experiment.

At 7 o'clock every morning from the date of flowering to maturity 40 bolls of each variety were collected from the three plots and taken to the laboratory for analysis. As the bolls were gathered they were put in small tightly stoppered glass jars to minimize the moisture loss. The length and diameter of 10 bolls were measured with a vernier caliper, and the length, width, and thickness of five seeds from each of five bolls were measured with a dissecting binocular fitted with a micrometer. All measurements were read to 0.1 mm. To facilitate measuring, the five seeds from each boll were placed on a glass slide, and after the length and width had been recorded each was turned on edge with a needle and the thickness measured near the center of the seed. Duplicate 100-seed samples were removed from the remaining bolls and dried to constant weight in an electric oven at 70° C.

During the afternoon of each day, from five days after flowering until maturity, enough bolls were collected from each plot to provide material for oil determinations.

The harvested bolls were placed on trays with screened bottoms to dry in the open air during the day. At night the trays were taken into the laboratory and the drying was continued in a current of air supplied by electric fans. When the bolls were well dried they were placed in thin cloth bags and hung in a well-aired room until threshed.

To determine the relation of planting date to yield and composition of the seed, four varieties of flax were planted at University Farm in 1929 and 1930 at successive 10-day intervals, beginning on May 1.

The four varieties used were Redwing (Minnesota accession No. 188), Bison (Minnesota accession No. 199), Buda (Minnesota accession No. 194), and Winona (Minnesota accession No. 182). The first two are the recommended varieties for the State. These varieties were grown in 3-row triplicated rod-row plots in 1929 and in 5-row triplicated plots in 1930. The center row was used in 1929 for yield and other plant characters and for study of the composition of the seed. The second row in the 5-row plots was harvested in 1930 for yield, and the fourth row was used for the study of plant characters.

The methods outlined by the Association of Official Agricultural Chemists (*2*) were used in the determination of the percentage and iodine number of the oil. The seed was finely ground in a special mill adapted to flaxseed, described in detail by Coleman and Fellows *(4)*. Duplicate 1-gram samples were used in the study of the daily increase in oil content. The extracted oil was dried in an electric oven at 80° C. for one and one-half hours, then weighed and redried for an additional half hour. The loss in weight during the final half hour was usually slight. Iodine numbers were determined on the oil secured from the ether extraction. The weighed oil was dissolved in 20 c c of chloroform and transferred to ground-glass stoppered flasks used for iodine absorption reaction. Wijis solution was used in all determinations of the iodine absorption number.

EXPERIMENTAL RESULTS

THE DAILY INCREASE IN SEED SIZE, BOLL SIZE, AND OIL CONTENT OF FLAX ON SOILS OF DIFFERENT PRODUCTIVITY

Daily measurements of the length, width, and thickness of the seed and the length and diameter of the boll were made with two varieties during the entire growth period from flowering to maturity. These measurements for flax grown on the different rotations show only a slight relation to soil productivity. The greatest difference occurred during the early part of the growth period, when the seed and boll dimensions were increasing most rapidly. After the approximate maximum size had been reached there was no consistent difference between the measurements of the bolls or seeds produced on the three plots. The daily measurements for the first seven days after flowering and the average of successive 7-day periods to maturity are given for both of the varieties in Table 1.

TABLE 1.—*Relation of soil productivity to daily and periodical increase in seed length, width, and thickness, and to boll length and width (millimeters), of Bison and Diadem flax grown on a 5 and 3 year rotation and a continuous cropping system*

SEED LENGTH

Days after flowering	Measurements of Bison grown in—			Measurements of Diadem grown in—		
	5-year rotation	3-year rotation	Continuous cropping	5-year rotation	3-year rotation	Continuous cropping
0	1.16	1.21	1.00	1.30	1.34	1.36
1	1.48	1.44	1.44	1.54	1.70	1.69
2	2.14	2.08	2.26	2.08	2.17	2.20
3	2.56	2.67	2.88	2.62	2.71	2.89
4	3.25	3.30	3.83	3.27	3.29	3.37
5	4.03	3.31	4.17	3.25	3.39	3.56
6	4.38	4.04	4.65	4.02	4.09	4.28
7	4.72	4.45	4.79	4.45	4.43	4.38
8–14	5.10	5.09	5.03	5.24	5.16	5.12
15–21	5.12	5.16	5.12	5.27	5.37	5.26
22–28	5.25	5.23	5.25	5.31	5.32	5.38
29–	5.15	5.04	4.97	5.26	5.24	5.28

SEED WIDTH

0	0.55	0.60	0.50	0.66	0.70	0.69
1	.75	.74	.74	.80	.91	.88
2	1.10	1.12	1.16	1.13	1.19	1.18
3	1.31	1.39	1.49	1.43	1.48	1.56
4	1.72	1.82	2.05	1.82	1.90	1.85
5	2.14	1.79	2.29	1.75	1.87	1.95
6	2.44	2.24	2.76	2.21	2.31	2.32
7	2.76	2.45	2.86	2.54	2.45	2.54
8–14	2.94	2.97	2.94	3.01	2.96	2.99
15–21	2.97	2.99	2.92	2.97	3.01	3.03
22–28	3.01	3.01	2.95	3.00	3.01	3.05
29–	2.88	2.72	2.65	2.80	2.79	2.89

SEED THICKNESS

0	0.38	0.37	0.34	0.44	0.40	0.44
1	.50	.50	.48	.52	.59	.54
2	.66	.62	.68	.68	.68	.66
3	.77	.78	.80	.80	.80	.86
4	.92	.91	1.10	.94	.93	.95
5	1.11	.98	1.02	.98	1.00	1.02
6	1.26	1.17	1.38	1.15	1.14	1.16
7	1.45	1.23	1.48	1.23	1.24	1.26
8–14	1.54	1.50	1.47	1.46	1.43	1.41
15–21	1.51	1.47	1.42	1.47	1.47	1.42
22–28	1.57	1.48	1.41	1.54	1.52	1.49
29–	1.45	1.24	1.14	1.43	1.39	1.38

BOLL LENGTH

0	3.35	3.48	3.16	4.05	4.14	4.32
1	3.98	3.87	3.51	4.18	4.31	4.55
2	4.59	4.48	4.20	4.97	5.28	4.80
3	5.03	4.98	4.92	5.33	5.54	5.69
4	5.60	5.88	5.66	6.03	6.34	6.48
5	6.02	5.50	5.89	5.59	5.77	6.02
6	6.59	6.20	6.76	6.50	6.50	6.77
7	7.00	6.68	6.85	6.76	7.00	6.94
8–14	7.52	7.65	7.39	7.99	7.97	8.18
15–21	7.54	7.73	7.57	8.10	8.54	8.41
22–28	7.69	7.77	7.62	8.24	8.44	8.42
29–	7.54	7.66	7.51	8.50	8.57	8.31

BOLL WIDTH

0	2.39	2.45	2.10	2.50	2.56	2.68
1	2.78	2.87	2.68	3.10	3.18	3.17
2	3.64	3.82	3.52	4.01	4.13	4.03
3	4.50	4.60	4.48	4.58	4.53	4.75
4	5.21	5.30	5.17	4.50	5.40	5.30
5	5.48	5.38	5.58	5.11	5.05	5.30
6	5.61	5.62	6.36	5.61	5.79	5.88
7	6.42	5.95	6.62	5.88	6.08	6.06
8–14	6.96	7.02	6.93	6.96	6.83	6.99
15–21	7.16	7.20	7.00	7.03	7.19	7.22
22–28	7.14	7.25	7.07	7.07	7.11	7.37
29–	7.16	7.18	7.05	7.11	7.03	7.21

Statistical comparisons of the daily boll and seed measurements have been made for the first 7-day period after flowering. By the use of Student's pairing method (*1*) and table of Z (*9*) the odds against the chance occurrence of differences as large or larger than those found have been determined. The mean differences and odds are summarized in Table 2.

TABLE 2.—*Statistical comparison of the mean difference in seed and boll size (millimeters) of two flax varieties, grown on soils of different productivity, with odds that differences are significant*

Measurement	Variety	5 and 3 year rotation		5-year rotation and continuous cropping		3-year rotation and continuous cropping	
		Mean difference	Odds	Mean difference	Odds	Mean difference	Odds
Seed length	Bison	0.0714	5:1	−0.1625	21:1	−0.2371	22:1
	Diadem	−.0738	196:1	−.1375	151:1	−.0763	36:1
Seed width	Bison	.0775	6:1	−.1225	70:1	−.1750	40:1
	Diadem	−.0588	47:1	−.0788	184:1	−.0200	45:1
Seed thickness	Bison	.0652	27:1	−.0538	22:1	−.1150	66:1
	Diadem	−.0038	1:1	−.0188	27:1	−.0150	6:1
Boll length	Bison	.1363	9:1	.1388	25:1	.0150	1:1
	Diadem	−.1838	1000:1	−.2700	302:1	−.0863	5:1
Boll diameter	Bison	.0050	1:1	−.0600	2:1	−.0650	2:1
	Diadem	−.0425	5:1	−.0988	20:1	−.0563	6:1

From the data given in Table 2 it is apparent that no consistent difference exists between the measurements of the bolls and seeds produced on the 3-year and 5-year rotation plots. Although the length and width of the seed and the length of the boll are significantly smaller for Diadem grown on the 5-year rotation plot, the differences (which are small) are in the opposite direction for Bison. This suggests a varietal response to soil productivity. A comparison of the seed and boll measurements from the 5-year rotation with the continuous cropping plot shows, with the exception of the boll length and diameter of Bison, a significant difference in favor of the continuously cropped plot. The comparisons of the boll and seed measurements from the 3-year rotation and the continuous cropping plot indicate a somewhat larger seed and boll size from the latter plot. The odds are not, however, as large as between the boll and seed measurements from the 5-year rotation and the continuous cropping plot.

The relation of soil productivity to the daily increase in dry weight of the seed is shown in Table 3.

TABLE 3.—*Relation of soil productivity to the average daily increase in dry weight (grams) of 100 seeds of Bison and Diadem flax grown in a 5 and 3 year rotation, and a continuous cropping system*

Days from flowering	Weight of 100 seeds from Bison grown in—			Weight of 100 seeds from Diadem grown in—		
	5-year rotation	3-year rotation	Continuous cropping	5-year rotation	3-year rotation	Continuous cropping
0	0.0080		0.0076	0.0104		0.0100
1	.0104	0.0108		.0144	0.0120	.0124
2	.0131	.0129	.0152	.0192	.0134	.0146
3	.0203	.0202	.0233	.0202	.0172	.0240
4	.0382	.0383	.0471	.0318	.0295	.0421
5	.0612	.0416	.0794	.0337	.0350	.0436
6	.0792	.0653	.1088	.0591	.0568	.0692
7	.1037	.0688	.1224	.0668	.0801	.0858
8	.1572	.1169	.1399	.1199	.0994	.1237
9		.1386	.1315	.1078	.1133	.1407
10	.1572	.1614	.1633	.1695	.1700	.1866
11	.1832	.1858	.2145	.1804	.1858	.1974
12	.2027	.1857	.2606	.1983	.2267	.2375
13	.2331	.2307	.2677	.2774		
14	.2357	.2475	.2872	.2681	.2634	.2610
15	.2575	.2693	.3108	.2515	.2428	.2836
16	.2860	.2860	.3492	.2798	.2989	.3067
17	.3022	.3080	.3934	.3157	.3207	.3182
18	.3459	.3756	.4262	.3200	.3716	
19	.4342	.4282	.5049	.3222	.3685	.3128
20		.4452	.5321	.3410	.4376	.3198
21	.4820	.4813	.5543	.3618	.4422	.3992
22	.5010	.5089	.5675	.4157	.4925	.4741
23	.5245	.5288	.5677	.4342	.4847	.4444
24	.5773	.5311	.5472	.5632	.5521	.5427
25	.5970	.5394	.5985	.5616		.5437
26	.5889	.5432		.5820		.5565
27	.5925	.5730	.5806	.6438	.6110	.5713
28	.6046	.5799		.6566	.5918	.5542
29	.6022	.5801	.5854	.6997	.6865	.6411
30	.5994	.6062		.6838	.6794	.6466
31	.5996	.6258	.5758	.6814	.6866	.6543
32	.5998	.6023		.7019	.7019	
33	.6518	.5809	.5790	.6657	.7150	.6284
34	.6516	.6188		.6780	.6720	.6281
35	.6392			.7025	.6992	.6438
36	.6581			.6622	.7092	.6356
37	.6760			.7296		
38				.7286		
39				.7018		

TABLE 4.—*Statistical comparisons of the mean difference in daily increase in dry weight (grams) of seed from soils of different productivity with odds that differences are significant*

Period of productivity	Variety	5 and 3 year rotation		5-year rotation and continuous cropping		3-year rotation and continuous cropping	
		Mean difference	Odds	Mean difference	Odds	Mean difference	Odds
First	Bison	0.007114	14:1	−0.021250	450:1	−0.027914	2,700:1
	Diadem	−.000336	1:1	−.012073	324:1	−.012700	1,374:1
Second	Bison	.002150	1:1	−.055663	470:1	−.061045	5,000:1
	Diadem	−.028873	22:1	.011000	4:1	.035460	243:1
Third	Bison	.023780	30:1	.024760	15:1	.004020	1:1
	Diadem	−.009325	5:1	.042200	10,000:1	.052857	4,000:1

Comparisons of seed weight of the flax grown in the three systems of cropping have been made for three periods of growth. The first period includes the first 15 days after flowering, the second from 15 days until the maximum seed size was reached, and the third from

maximum seed size to maturity. The approximate maximum seed size was reached on the twenty-fourth day after flowering for Bison and on the twenty-eighth day for Diadem. The mean difference and the odds derived from student's pairing method and tables of Z are given in Table 4.

The results obtained indicate approximately the same response to soil productivity as was shown by the seed and boll measurements. (Table 1.) The differences in seed weight of flax produced on the 3 and 5 year rotation plots are not significant, but the odds are great that during the first two growth periods the seed weight obtained from either of the rotation plots is less than that obtained from the continuous cropping plot, indicating that the seed developed more rapidly on the soil of lowest productivity. During the final growth period when the increase in seed weight was slight, the above relation was reversed for Diadem only, and the odds are great that the seed weight is less from the continuous cropping system than from either of the rotations. The difference in varietal response during the final growth period suggests that Bison is better suited to soil of low productivity than Diadem.

Determinations of the daily increase in oil content were begun at five days after flowering for the 3-year rotation and at seven days after flowering for the 5-year rotation and the continuous cropping system. At the 5-day stage there was considerable difficulty in separating the very small seeds from the threshed bolls. The percentage of oil from Bison only is given in Table 5. These results, secured at frequent periods of development, show no consistent differences in response to soil productivity for the different cropping systems during the period of oil formation. Statistical comparisons of the oil content made from the twenty-third day to maturity between the flax produced on the 5 and 3 year rotation and the 5-year rotation and continuous cropping plots give a mean difference in favor of the 5-year rotation of 0.56 per cent and 0.87 per cent oil, respectively, with odds of 6:1 and 21:1 that these differences are significant. The comparison between the oil content of the seed from the 3-year rotation and continuous cropping plots gives a mean difference in favor of the former of 0.42 per cent oil. The odds are only 4:1 against the occurrence of differences as large or larger due to errors of random sampling.

TABLE 5.—*Relation of soil productivity to the daily increase in percentage oil content of Bison flax based on the dry weight of the seed*

Days from flowering	Percentage oil content of flaxseed			Days from flowering	Percentage oil content of flaxseed		
	5-year rotation	3-year rotation	Continuous cropping		5-year rotation	3-year rotation	Continuous cropping
5		9.7		19	36.0	35.7	37.7
7	14.3	13.3	16.3	21	37.5	37.7	37.7
8		18.2	15.0	23	38.0	39.1	39.1
9	14.3	18.8		25	40.5	39.5	38.7
10			21.4	27	39.9	39.1	38.4
11	24.5	19.0		30	39.7	39.1	38.8
13	26.6	23.6	31.5	33	39.7	38.2	38.9
15	29.1	32.1	33.8	36	39.3		38.0
17	35.1	33.8	36.0				

Varietal differences in the rate of development of seed and boll size, dry weight, and oil content of the seed are shown by the average results from the three cropping systems. Such an average is justifiable from the statistical comparisons previously made. This average also shows more accurately daily changes in size and composition of the seed throughout the entire growth period. The growth curves (fig. 1) from the averaged results show that the length, width, and thickness of the seed increased rapidly, reaching an approximate maximum at nine days after flowering. From this stage until shortly before ma-

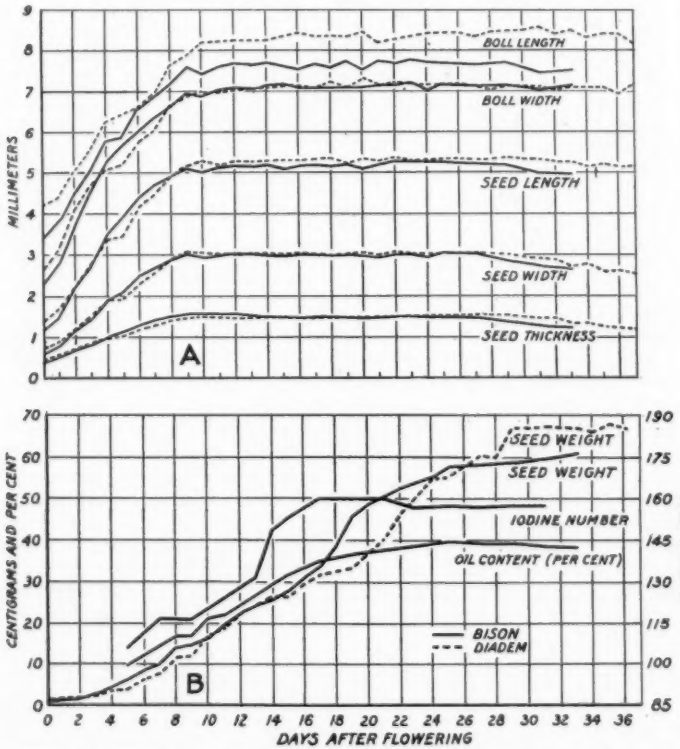

FIGURE 1.—Average daily seed and boll measurements, (A) dry weight of 100 seeds, and the percentage and iodine number (B) of the oil of Bison and Diadem flax

turity there was no great change in seed size. Coincident with the decrease in moisture content with approaching maturity, the measurements show a slight but consistent decrease. Throughout the entire growth period there was a remarkably close agreement in the daily development of the two varieties, which probably indicates that the flax varieties used are quite similar physiologically although different morphologically. The close agreement of these results with those secured by Dillman (*5*) with the variety Rio (C. I. 280) gives further evidence for this conclusion. Since the maximum seed size is reached

at an early stage of development, it is quite likely that environmental influences have only small opportunity to become manifest.

The daily increase in length and diameter of the boll follows very closely that of seed size, reaching a maximum for both varieties at nine days after flowering. From this stage until shortly before maturity there is no appreciable change in size. The lowering of the moisture content with maturity resulted in a slight reduction in boll size.

The daily increase in dry weight of the seed progresses at a fairly uniform rate until approximately 24 days after flowering for Bison and 28 days after flowering for Diadem. This difference of four days represents the difference in number of days from flowering to maturity of the two varieties, the variety Diadem maturing somewhat later than Bison. After the seeds reached their maximum weight there was no great increase to maturity. These results are again in close agreement with those secured by Dillman (*5*).

The average oil content shows a fairly uniform increase from 5 to 25 days after flowering, followed by a slight but consistent decrease until maturity. The initial oil content is much higher than was found by Dillman (*5*). This difference may be explained in part by the difference in analytical method used. Dillman determined the oil content by the refractive index method developed by Coleman and Fellows (*4*). The accuracy of this method is dependent on a constant initial refractive index of the oil. Since the increase in oil content is positively correlated with an increase in refractive index, it is apparent that an initial refractive index lower than the accepted standard will give a lower oil content than that which actually exists in a given sample. Unpublished data furnished by Anderson [5] show a high positive correlation between the iodine value and the refractive index. Since the iodine value at early stages of development is much lower than at later stages, it is quite likely that the refractive index of the oil is likewise lower than the standard value. Oil determination by such methods may therefore give erroneous results in this type of study. The results secured during the later stages of development agree very closely with those reported by other investigators.

Determination of the iodine absorption number of the oil was made on seed of Bison obtained from the 3-year rotation plot. The results given in Figure 1 and Table 6 suggest that the fatty acids which make up the oil at early stages of development are more saturated than those at later stages. The progressive change in degree of saturation is completed at 17 days after flowering and remains constant for a short time, then decreases from this point to maturity.

TABLE 6.—*Iodine absorption numbers of Bison flax from the 3-year rotation system on various days after flowering*

Days after flowering	Iodine No.	Days after flowering	Iodine No.	Days after flowering	Iodine No.	Days after flowering	Iodine No.
5	106	13	131	19	160	27	157
7	116	14	148	21	160	30	158
9	116	15	153	23	158	33	158
11	124	17	160	25	158		

[5] Anderson, R. E., chemist, Archer-Daniels-Midland Co.

These results confirm the conclusion reached by Ivanow (*10*), and indicate that in the synthesis of the oils in flaxseed the saturated fatty acids are formed first. These are then progressively transformed to acids possessing a greater degree of unsaturation. The view that fatty acids are synthesized from glucose is generally accepted at the present time, although the exact intermediate products are not known. During the ether extraction of seed at the period of five to eight days after flowering, traces of resinous substances were isolated from the extracted oil. Although these substances were originally ether soluble, it was impossible to redissolve them in ether after the ether had been evaporated. Preliminary studies indicate that the seed contained from 0.3 to 0.7 per cent of this insoluble fraction. It is quite possible that these substances may be intermediate condensation products in the synthesis of fatty acids from glucose. No traces of this product were found after the 9-day stage.

THE RELATION OF DELAYED PLANTING TO YIELD AND COMPOSITION OF THE SEED

Extensive investigations at University Farm, St. Paul, with several standard varieties have shown that delayed planting of flax generally results in a considerable reduction in yield of seed. Similar results have been secured by investigators at other experiment stations. The reason for such reductions in yield has not always been apparent, since in most instances the difference in percentage of full stand at each planting date has not been sufficient to bring about wide yield differences. During 1929 and 1930 detailed studies were made to determine whether the reduced yield could be explained on the basis of the average number of seeds per boll and the average number of bolls per plant. The determinations were made on four flax varieties planted in replicated rod-row plots at five successive 10-day intervals beginning on May 1. In 1930 an attempt was made to establish a uniform stand at each planting date by thinning the plants when about 1 foot tall to a distance of 1 inch apart within the row. This distance permitted thinning for the first three dates, but a poor stand gave for the last two plantings an average spacing greater than for the first three. In 1929 the plants were not thinned. The stand, however, was approximately uniform for all dates.

The average number of bolls per plant was determined from counts of approximately 400 plants uniformly distributed among three replicates. Between 400 and 500 bolls selected at random from the plants were threshed individually to determine the average number of seeds per boll.

The results secured from each of the varieties for five dates of planting show a remarkably close agreement in 1929 between the decrease in yield and the reduction in number of seeds per boll. (Table 7.) Of the four varieties, Redwing and Winona show the least reduction in yield and in seeds per boll as a result of delayed planting. Although the average number of bolls per plant was greatly decreased by delayed planting, its relation to yield is not as striking as is the number of seeds per boll.

TABLE 7.—*Relation of delayed planting to the yield of seed, average number of seeds per boll, and average number of bolls per plant of four flax varieties planted at successive 10-day intervals*

[The yield data are given in percentage of that obtained from the May 1 planting]

SEED YIELD IN PERCENTAGE

Variety	Planting date, 1929					Planting date, 1930				
	May 1	May 10	May 20	June 1	June 10	May 1	May 10	May 20	June 1	June 10
Redwing	100	99.5	77.3	19.3	3.9	100	78.8	58.0	35.9	13.9
Bison	100	72.2	66.1	8.7	2.6	100	69.3	38.0	20.0	4.4
Buda	100	46.2	41.8	6.6	2.2	100	64.9	33.6	16.0	5.3
Winona	100	80.3	73.2	23.6	10.2	100	92.3	63.2	51.6	21.3
Weighted average	100	80.0	68.1	16.3	5.2	100	76.2	49.2	21.0	11.0

NUMBER OF SEEDS PER BOLL

Redwing	7.80	7.79	7.10	3.28	0.83	8.31	8.77	7.63	5.98	5.54
Bison	6.24	6.00	3.92	.65	.43	7.29	6.44	4.49	2.91	4.90
Buda	6.82	5.66	4.99	1.07	.27	6.74	4.42	4.37	3.20	4.18
Winona	7.95	7.37	6.89	3.00	1.26	7.49	7.40	6.88	5.37	5.20
Average	7.20	6.71	5.73	2.00	.70	7.46	6.76	5.84	4.37	4.96
Per cent	100	93.2	79.6	27.8	9.7	100	90.6	78.3	58.6	66.5

NUMBER OF BOLLS PER PLANT

Redwing	11.22	11.47	7.16	6.97	5.69	15.52	15.86	9.73	8.92	10.84
Bison	15.93	14.27	10.63	7.13	5.75	20.36	15.94	10.24	10.15	4.48
Buda	15.00	18.32	10.53	7.28	4.18	16.86	17.29	9.90	10.98	4.84
Winona	17.28	15.02	11.36	8.57	9.07	19.02	15.76	9.02	14.48	10.89
Average	14.86	14.77	9.92	7.49	6.17	17.94	16.21	9.72	11.13	7.76
Per cent	100	99.4	66.8	50.4	41.5	100	90.4	54.2	62.0	43.3

In 1930 the relation between yield at the different planting dates and the number of seeds per boll and bolls per plant is not as close as in the previous year. This is true especially for the last two dates, where the poor stand probably influenced the results. The four varieties responded in nearly the same way as in the previous year's study, Redwing and Winona being less influenced by delayed planting than Bison or Buda. The average results for the four varieties given in percentage of the yield obtained from the May 1 date of planting are shown for each of the two years in Figure 2.

Although the two years' study has shown a marked reduction in the number of seeds per boll as a result of delayed planting, the exact reason for this response is not known. Pollen sterility resulting from high temperature at flowering time is not a satisfactory explanation, since rudimentary seeds were found to be present in the mature bolls, indicating that fertilization had taken place. It is quite possible that desiccation resulting from high temperatures during the early period of seed formation may have injured the developing ovules. The very immature seeds have a high moisture content (80–83 per cent during the first 12 days in 1929), and moisture loss through the thin wall of the inclosing boll may have reduced their water content to a point where further development could not take place.

The influence of delayed planting on the oil content of the seed was determined from four varieties in 1929 and two varieties in 1930. The iodine number of the extracted oil was made on the 1930 crop. Table 8 shows that delayed planting resulted in a very slight reduction in oil content in 1929, the reduction in Bison being somewhat greater than in the other varieties. In 1930, however, there was no consistent change in either of the two varieties used. These results are in close agreement with those reported by other investigators (*3*, *9*).

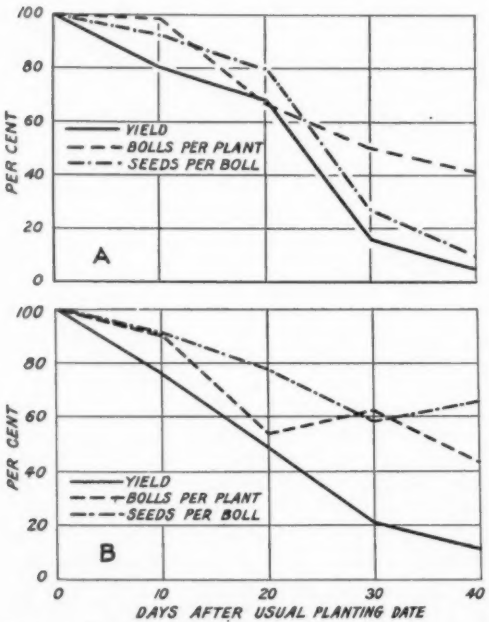

FIGURE 2.—The relation of delayed planting to the yield of seed, number of bolls per plant, and number of seeds per boll in 1929 (A) and 1930 (B). The data are given in percentages of those obtained from the May 1, or usual, planting date

The data from the study of the iodine number of the oil from the 1930 crop show a consistent reduction for Bison for the first four dates but no significant change for Redwing. Both varieties had a higher iodine number for the last date of planting than for the other dates. The seed from this planting was not fully mature when harvested, and therefore the results from this date of planting are not comparable with the others. The studies made on the oil from seed at frequent stages of development gave a somewhat lower iodine number for the fully mature seed than for immature seed at 17 days after flowering. The results secured by Bushey, Puhr, and Hume (*3*) likewise show a lowering of the iodine number with progressive maturity. These results suggest that the high iodine number of the seed from the last date of planting was due to its immaturity.

The results of these investigations, together with those previously reported, show conclusively that oil content is less influenced by environmental changes than are the other plant characters studied. The maintenance of uniformity in oil content while other characters were changed by delayed planting suggests that the composition of flaxseed is a fairly constant heritable character. The uniformity in oil content does not, however, justify late planting of flax, inasmuch as this practice results in a lower yield of seed.

TABLE 8.—*Relation of delayed planting to the percentage of oil, iodine number, and average number of days from full bloom to maturity of flax varieties planted at successive 10-day intervals after May 1*

PERCENTAGE OIL

Variety	Planting date, 1929					Planting date, 1930				
	May 1	May 10	May 20	June 1	June 10	May 1	May 10	May 20	June 1	June 10
Redwing	36.4	36.7	36.0	35.7	34.6	34.7	35.1	35.4	34.7	35.1
Bison	37.8	37.3	37.1	36.4	35.5	36.1	36.7	36.4	36.1	36.7
Buda	36.7	36.8	35.3	35.3	35.0					
Winona	34.4	34.5	34.3	34.2	33.8					
Average	36.3	36.3	35.7	35.4	34.7	35.4	35.9	35.9	35.4	35.9

IODINE NUMBER

Redwing						175.2	176.4	175.3	174.8	181.0
Bison						164.6	159.4	158.2	156.4	172.2

DAYS FROM FULL BLOOM TO RIPE

Redwing	46	42	37	32	35	32	26	30	34	
Bison	42	42	36	35	32	32	30	30	28	
Buda	42	42	38	35	34	29	31	29	30	
Winona	42	43	38	32	32	33	29	29	34	
Average	43	42	37	34	33	32	29	29	32	

SUMMARY

The experimental results reported here include two phases of a study with flax—(1) the rate of development of the seed, boll, and oil on soils of different productivity, and (2) the influence of delayed planting on yield and composition of the seed.

In the first phase of the study two varieties of flax, Bison (Minnesota accession No. 199) and Diadem (Minnesota accession No. 197), were grown at University Farm, St. Paul, Minn., on a portion of the wheat plots of a 5 and 3 year rotation and on a plot continuously cropped to wheat.

Daily measurements were made of the length, width, and thickness of the seed, length and diameter of the boll, and dry weight of the seed of both varieties. The rate of development of the oil content was studied only with Bison. The iodine number was determined on the seed obtained from Bison grown on the 3-year-rotation plot.

The daily seed and boll measurements indicate a more rapid rate of growth during the first week after flowering on the soil of lowest productivity (the continuous cropping plot). After the maximum seed and boll size was reached there was no consistent difference in size of seed or boll on either of the plots used.

The measurement of the daily increase in dry weight of the seed gave the same type of response on soils of different productivity during the period of most rapid increase in seed weight. From this period to maturity (approximately seven days) the seed of Diadem grown on the continuous-cropping plot was found to be significantly smaller than from the 3 or 5 year rotation plots. The seed weight

of Bison during a similar period was practically the same from each of the plots, which suggests a varietal response to soil fertility, Bison probably being better suited than Diadem to soil of low productiveness.

Varietal difference in rate of development of seed and boll and the dry weight of the seed were studied from the averages from the three plots. The growth curves show that the length, width, and thickness of the seed and the length and diameter of the boll increased rapidly, reaching an approximate maximum size for both varieties at nine days after flowering, remained fairly constant for a considerable period, then decreased slightly with complete maturity.

The daily increase in dry weight of the seed progressed at a fairly uniform rate until 24 days after flowering for Bison and 28 days after flowering for Diadem, then increased slightly to complete maturity. The results of the measurements show that the growth rate of both varieties are nearly identical.

The average oil content of Bison, studied at 2-day intervals, showed a fairly uniform increase from 5 days to 25 days after flowering, followed by a slight decrease to maturity.

The iodine absorption number made at frequent periods on the seed of Bison from the 3-year rotation suggests that the saturated fatty acids are formed first in the synthesis of oil, but are progressively transformed to acids with a greater degree of unsaturation. The iodine numbers increased rapidly from 5 days to 17 days after flowering, remained fairly constant for a short period, then decreased slightly to complete maturity.

In the second part of the study, which dealt with the influence of delayed planting on the yield and composition of the seed, four standard varieties were grown during 1929 and 1930 in replicated rod-row plots and were sown at five successive 10-day intervals beginning on May 1. From these varieties the yield of seed, average number of seeds per boll, average number of bolls per plant, percentage of oil, and the iodine absorption number of the oil were determined from each planting date.

The results of this phase of the study show that the yield of seed is greatly reduced as a result of delayed planting. The reduction in the number of seeds per boll and the number of bolls per plant followed very closely the reduction in yield, which may explain the lowered yield from late-planted flax. The reduction in the number of seeds per boll could not be attributed to lack of fertilization of the flower, since rudimentary seeds were present in the ovary. High temperatures may have been lethal to the developing seed at an early stage of growth when its moisture content was very high.

The analysis of the seed showed a very slight reduction in oil content with delayed planting in 1929, but no consistent differences in 1930. The iodine number of the oil determined from two varieties in 1930 indicated a slight decrease with delayed planting for Bison but no significant change for Redwing.

The results of this study suggest a difference in varietal response to delayed planting. Of the four varieties tested, Redwing and Winona showed less reduction in yield than Bison and Buda.

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